

Enteric Protozoal Infections among Children with Cancers Receiving Chemotherapy

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Abstract:

Objectives: To detect enteric protozoal species in stool samples of children affected by malignancies who are receiving chemotherapy compared to healthy children and to investigate differences in the infection rates among different cancer types.

Materials and Methods: This study included 100 children diagnosed with malignancy under chemotherapy attended Ibn-Al-Atheer Teaching Hospital during the period from December 2013 to June 2014. The study also included 50 apparently healthy children as a control group who were age and gender matched to the cancer patients. Children were already diagnosed as having malignant disease by consultant oncologists and receiving chemotherapy. A Questionnaire Form was completed for each child. Stool samples were taken from patients and controls to detect protozoal infections. The stool was tested by direct wet mount preparation, iodine staining, and concentration methods, hot modified Ziehl-Neelson staining method was also used to detect the acid-fast oocysts of *Cryptosporidium*.

Results: Enteric protozoal infection was detected in 60 (60%) of patients compared to 5(10%) of controls ($p < 0.05$). Four protozoal species were observed among patients included *G. lamblia* representing (24%), *C. parvum* (19%), *E. histolytica/E. dispar* (5%), and *B. species* (5%). Whereas in the control group, included *G. lamblia* (6%), *C. parvum* (2%), and *B. species* (2%). The highest infection rate was found among children with lymphoma (77.27%), followed by (62.26%) and (40%) among patients with leukemia and solid tumors, respectively ($p > 0.05$).

Conclusions: The incidence of enteric protozoal infections is higher among cancer children than healthy group and dominates by *Giardia lamblia* and *Cryptosporidium parvum*, but other species including *E. histolytica/ E. dispar* and *B. hominis* are also found. Patients with lymphohematopoietic malignancies show the highest infection rates.

Introduction

Intestinal parasitic infections are globally endemic and have been described as constituting the greatest single worldwide cause of illness and disease (1). In Iraq, the hot weather, humidity, suitable habitat, and poor hygiene specially in rural areas aggravate the spread of such infections among the population (2,3). It had been noticed that intestinal protozoan parasites remain the most commonly encountered parasitic diseases and continue to cause significant morbidity and mortality (4). All ages are liable to protozoal infections but children are located at the top of the list (5). Such infections have been variably reported among immune compromised people especially those with malignancy and/or patients submitted to immunosuppressive therapy (6,7). Immunosuppressed patients are prone to opportunistic infections that cause auto limited diarrhea in immunocompetent children, while they cause serious recurrent diseases in immunocompromised. Such infections may be caused by *Cryptosporidium*, *Microsporidia*, and *Blastocystis* spp. (8,9). Furthermore, pathogenic protozoan parasites commonly associated with gastrointestinal disease can affect immunodeficient children, for example, *Giardia lamblia*, *Entamoeba histolytica*, and rarely *Balantidium coli* (10,8). Immunosuppressed individuals can easily acquire infections, but it is difficult to eradicate them since they result in severe and disseminated disease rather than localized infection (4).

Materials and Methods

The present study was carried out in the Diagnostic Laboratory at the Department of Microbiology, College of Medicine, University of Mosul. The data of this study

were collected during the period from December 2013 up to June 2014 at Ibn-Al-Atheer Teaching Hospital in Mosul, Iraq. Two groups were included in the study, first group was composed of 100 children diagnosed previously as having malignancy and under chemotherapy (60 male, 40female). Their ages ranged from 1-12 years, and mean $\pm$ standard deviation in patients 5.67 ± 2.84 . Fifty had acute lymphoblastic leukemia, 3 had acute myeloid leukemia, 16 had Non-Hodgkin lymphoma, 6 had Hodgkin's lymphoma, and 25 patients with solid malignancies. A special data form for each child was provided. The second group involved 50 apparently healthy children (28 male, 22 female), between the ages of 1-12 years, and mean $\pm$ standard deviation was 5.6 ± 3.5 .

Stool sample collection, handling, storage and examination

Faecal specimens were collected freshly and preserved in a dry, clean, sterile, tightly covered, wide mouth plastic containers. Direct wet mount method using normal saline and lugol's iodine solution and concentration technique were carried out for all the samples to detect the non-acid fast protozoa. Hot modified Ziehl-Neelson method was used to detect the acid-fast *Cryptosporidium parvum* oocyst (11).

Statistical analysis

The data were statistically analyzed by using tables and pie. Standard statistical methods were used to describe the results of the study : mean, standard deviation (SD), number and percentage. Two - proportions test was performed using p- value of (0.05), Minitab version -7

program was used. The statistical results were considered significant at $p \leq 0.05$ (12).

Results & Discussion

Children were divided into three age groups (toddlers, preschool, school) (Fig.1). Among different age groups, toddlers had the greatest proportion of acquiring intestinal protozoal infection in both patients and control groups that were (21/26, 80.76%) and (2/10, 20%) respectively. There was a significant difference in the infection rate between each age group of patients and controls with ($p < 0.05$), according to the two proportions test (Table 1). This result is in agreement with previous studies in which toddlers (1-4 years old) showed the highest rate of intestinal protozoal infection (70%) (13). These findings may be attributed to the higher exposure rate of children in this age group, as they are careless with hygiene measures. Whereas other studies detected that school age children (6-12 years) had the greatest proportion of acquiring protozoal infection (47%) (14). The rate of infection in male was higher (63.33%) than female (36.66%) (Fig.2), but this difference was non-significant ($p > 0.05$). This result was in agreement with previous studies done in Dohuk city and Basra city in Iraq (15,16). This may be due to the fact that males at this age are more exposed to infections because they are more active than females and integrated in the environment (15).

Sixty children with malignancy (60%) and 5 (10%) of the healthy control were positive for protozoa in their stool samples. The difference was statistically significant ($p = 0.000$) (Table 2). This result is similar to earlier studies done in Iraq and other countries by Al-Sherrefat N.S.S.,2002, Al-Sadoon M. A.,2012, Menon *et al.*,1999, and Abaza *et al.*,1995 which detect a higher infection rate among cancer children than healthy group (11,17,18,19).

Out of the 60 positive samples of the patients, four protozoal species were detected, of them *G. lamblia* representing (24%), *C. parvum* (19%), *E. histolytica* (5%), and *B. species*. (5%), compared to an incidence of 14%, 4%, 2%, and 26% for the same protozoa respectively, in the study of Aksoy *et al.* (14). Our study showed higher rates of infection than other similar studies which could be due to the greatest group of lymphohematopoietic malignancy over other types of neoplasms, all patients were under immunocompromising effect of chemotherapy, the use of different staining methods to detect protozoal infection, and the higher number of patients included in the study. Moreover, mixed infections were found include *G. lamblia* & *C. parvum* in a rate of (5%) and *E. histolytica* & *C. parvum* was (2%). From the control group, 5 positive samples were identified including *G. lamblia* (6%), *C. parvum* (2%), and *B. species*. (2%) (Table 2).

The highest percentage of protozoal infection was recorded among patients with lymphoma (77.27%) followed by (62.26%) among patients with leukemia and (40%) among patients with solid tumors, this difference was not significant ($p > 0.05$) (Table 3). These results were similar to that of Mahdi *et al.* who reported infection rates of 60.86%, 46.66%, and 44.44% among patients with lymphoma, leukemia, and solid tumors respectively (20). The higher incidence rate of enteric

protozoal infections among patients with lymphohematopoietic malignancies could be due to their effects on immunity mechanisms that include mucosal membrane, skin, quantitative and qualitative defect of granulocytes and macrophages (21).

In Conclusion, children affected by malignancies and under treatment by chemotherapy show higher liability to get protozoal infections than healthy children. The higher infection rate is seen among lymphoma patients. Enteric protozoan infections are dominated by *Giardia lamblia* and *Cryptosporidium parvum*, but other species including *E. histolytica*/ *E. dispar* and *B. species* are also found. Therefore, intestinal protozoan infections should be considered during diagnosis of cancer children in order to treat them and prevent their complications.

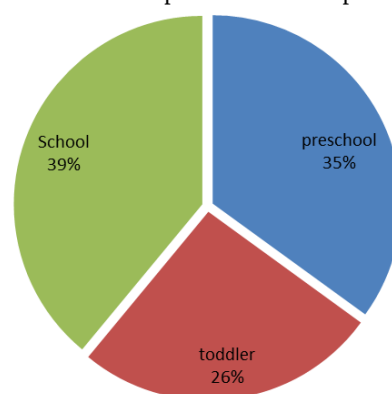


Figure 1: The frequency of ages of children with malignancy

Table (1): Distribution of enteric protozoal infections in the patients and controls according to age group

Age group	Patients			Controls		
	E	+	%	E	+	%
Toddlers >1-4 years	26	21	80.76%	10	2	20%
Preschool >4-6 years	35	15	42.85%	20	1	5%
School >6-12 years	39	24	61.5%	20	2	10%
Total	100			50		

E=Examined number, (+)= number of infected patients
A significant difference found between each age group from patients and controls with ($p < 0.05$), according to the two proportion test

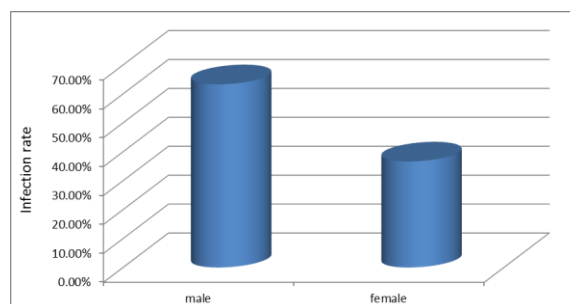


Figure 2 : Enteric protozoan infection rates according to the patients gender

Table (2) : Species of intestinal protozoa among patients and controls

Protozoan species	Patients		Controls	
	No.	%	No.	%
<i>G. lamblia</i>	24	24%	3	6%
<i>C. parvum</i>	19	19%	1	2%
<i>E. histolytica/ E. dispar</i>	5	5%	0	0
<i>B. species</i>	5	5%	1	2%
<i>C. parvum</i> + <i>G. lamblia</i>	5	5%	0	0
<i>C. parvum</i> + <i>E. histolytica</i>	2	2%	0	0
Total	60	60%	5	10%

No.= Number of samples

A significant difference in the infection rate was detected according to the two proportion test, with ($p=0.000$)

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Table (3): Distribution of enteric protozoan infections according to their species, among children with different malignant diseases

Types of malignant cases	Examined No.	Positive protozoan infection	
		No.	%
Lymphoma	22	17	77.72
Leukemia	53	33	62.26
Non-Lymphoid Solid tumors	25	10	40
Total	100	60	

No= Number

The difference was non-significant with ($p>0.05$), according to the two proportions test

اصابات الحيوانات الاوالي المعوية لدى الاطفال المصابين بالسرطان الذين يتلقون العلاج الكيميائي

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الملخص

الأهداف: التحري عن انواع الحيوانات الاوالي المعوية في عينات خروج الاطفال المصابين بالاورام الخبيثة الذين يتلقون العلاج الكيميائي مقارنة بالاطفال الاصحاء، و ايجاد الاختلاف بمعدلات الإصابة بين انواع السرطان المختلفة.

عينات الدراسة وطرائق العمل : هذه الدراسة شملت 100 طفلا مصابا بالسرطان ويتلقون العلاج الكيميائي الذين راجعوا مستشفى ابن الاثير التعليمي، وخلال الفترة من كانون الاول 2013 الى حزيران 2014ز كذلك شملت الدراسة 50 طفلا سليما يمثلون مجموعة السيطرة والذين كانوا مطابقين في اعمارهم واجناسهم للمرضى، وبعد اخذ معلومات كاملة من كل مريض كان قد شخص مسبقا بالسرطان من قبل اختصاصي الاورام تم ملئ استمارة الاستطلاع الخاصة بهذه الدراسة ثم تم اخذ عينة خروج من كل من المرضى والاصحاء، حيث تم فحص العينات بواسطة الفحص المجهرى المباشر، صبغة الايودين، طريقة التركيز وكما استخدمت صبغة (Ziehl-Neelson) لتشخيص البيوض المتكيسة للـ *Cryptosporidium*.

النتائج: اصابات الحيوانات الوالي المعوية كانت اعلى لدى المرضى 60 (60%) مقارنة بالاصحاء 5 (10%) وقد كان هذا الختلاف معنويا من الناحية الاحصائية ($p < 0.05$). هريعة انواع من الحيوانات الاوالي لوحظت لدى المرضى وشملت *G. lamblia* representing (24%), *C. parvum* (19%), *E. histolytica/E. dispar* (5%), and *B. hominis* (5%)، بينما في مجموعة الصحاء شملت *G. lamblia* (6%), *C. parvum* (2%), and *B. hominis* (2%). اعلى نسبة اصابة وجدت بين الاطفال المصابين بالورام اللمفية (77.27%) تلتها (62.26%) لدى الاطفال المصابين بابيضاض الدم، ثم (40%) لذوي الاورام الاخرى.

الاستنتاجات : انتشار الاصابات الطفيلية المعوية كان اعلى لدى الاطفال المصابين بالسرطان منه لدى مجموعة الاصحاء. واتضح ان (*G. lamblia* & *C. parvum*) هما النوعان الاكثر شيوعا لكن توجد انواع اخرى تشمل (*E. histolytica/E. dispar* & *Blastocystis*) (species) . ان اعلى نسبة اصابة تظهر بين الاطفال المصابين بالاورام اللمفية يليها ابيضاض الدم ثم انواع الاورام الاخرى.