PROTOZOAL ETIOLOGY OF ACUTE AND PERSISTENT DIARRHEA IN CHILDREN IN AL-SOWERA CITY IN IRAQ[+]

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Abstract

The aim of this study was to investigate the protozoal causes and their prevalence rate among diarrheic children with an age range of 0-60 months. Six hundred stool samples were collected from the General Hospital of Al-Sowera (the Wasit governorate-Iraq), and were analyzed for the presence of pathogenic protozoa. The prevalence of pathogenic protozoa was 208 cases representing 34.6% of the total diarrheic stool specimens. The № of infected cases with *Endameba histolytica* was 96 which represent the highest percentage (16%) among the total diarrheic stool specimens, followed by *Giardia lamblia* which was 66 cases (11%) and *Cryptosporidium parvum* which was 14 cases (2.3%). No significant variation in the prevalence rate between males and females was noticed (P= or< 0.0001), however, a significant variation in the prevalence rate according to the age groups was recorded (P > 0.01) in which the highest rate was noticed among the age group of 0-20 months. All non-pathogenic intestinal protozoa that were detected in the stool samples were not included in the results of this study.

المستخلص

استهدفت هذه الدراسة تقصي انتشار الأوالي المعوية المسببة للاسهال بين الأطفال بعمر ٠-٦٠ شهرا لكلا الجنسين في عينات البراز التي جمعت من حالات الأسهال للاطفال المرضى في مستشفى الصويرة العام لفترة من كانون الثاني (يناير) الى كانون الأول (ديسمبر) للعام ٢٠٠٤. جمعت٦٠٠ عينة براز كان منها٢٠٨حالة سبب الأسهال فيها يعود الى الأوالي المعوية والتي شكلت نسبة ٣٤,٦%من مجموع الحالات. اعلى نسبة اصابة كانت بالمتحولة الحالة للنسيج يليها الأصابة بالجارديا اللامبيليا واقل نسبة كانت بالابواغ الخبيئة (١٦%و ١١% و٢,٣% على التوالي). لم يكن هناك فرق معنوي للاصابة بين الجنسين من المصابين ولكن كان هناك فرق معنوي للاصابة بين المجاميع العمرية حيث سجلت اعلى نسبة اصابة في الفئة العمرية ٠-٢٠ شهرا.

Introduction

Diarrhea among children can be caused by many infectious agents like bacterial, parasitic, viral and fungal, and other non-infectious agents like nutritional and malignancies. Acute infective diarrhea that results from parasitic infections is very common in developing countries and is highly related to the poor sanitations and hygienic measures, as well as the socio-economic and educational levels of

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population [1] and usually associated with ill health and premature death mainly , among children [2].

Many studies were conducted in Iraq which dealt with types, etiology and geographical distribution of parasitic infections and their consequent acute infective diarrhea [3,4,5,6,7,8,9,10].

Protozoan parasites are one of the major causes of acute infective and persistent diarrhea, according to many previous studies, and, among those protozoa, the *Entameba histolytica* is the most important in its devastating effect and endemicity in many tropical and subtropical areas in the world [11]. About 500 millions people are infected world-wide [12] and up to 100,000 deaths per year have been attributed to complications of amoebiasis, notably amoebic liver abscess [13]. Amoebic dysentery is the most common form of amoebiasis caused by *E. histolytica* in children which is highly transmissible through fecal-oral contamination and food or drinkable fluids contaminated with the cyst form of the parasite [14, 15]. The infection may be acquired from a non-human origin as cats and dogs. Human is the normal reservoir of this protozoon that can transmit the infection to these animals [16].

The other protozoa that considered as one of the most world wide distributed parasite and mainly associated with infective diarrhea among children is *Giardia lamblia*. Giardiasis in children is highly endemic in tropical areas [17] which can cause diarrhea, abdominal pain, flatulence, vomiting, weight loss, mal-absorption with lactose [18], as well as some destructive effects on the small intestine villus, reduced disaccharide activity and absorption of electrolytes and nutrients [19, 20]. Some reports mentioned about the possibility of some complications which may occurs due to infection with *G. lamblia* like fatigue, arthritis and ocular lesions [21, 22] .

Direct child to child fecal oral route is the main possible way for the transmission of infection in endemic situation [23].

Diarrhea may also be caused by human intestinal coccidian including *Isospora beli*, *Cryptosporidium parvum* and *Cyclospora cayetanensis*. They have a world wide distribution particularly in tropical and developing countries. The infection is transmitted by fecal oral route with infective oocyst being ingested, however, many water-borne out-breaks of *C. parvum* have been reported in many tropical areas [24] in which this protozoa was considered as an important cause of diarrhea in young children and toddlers. Persons with abnormal immune system, particularly those with AIDS, the infection with *C. parvum* can cause acute and often fatal diarrhea [25-27].

Person to person transmission of the infection is common and animal hosts like calves, lambs, goats and pets are also important source of human infection [28]. An episode of acute diarrhea was defined as three or more loose bowel movement per day over 72 hours period. When diarrhea persisted for more that 14 days, it was defined as persistent diarrhea [29].

Materials and Methods

Six hundred stool specimens had been collected from children with ages from 0-60 months of both sexes with diarrhea (persistent or acute) who were either out-patients or in-patients from the General Hospital of Al-Sowera (Wasit governorate-Iraq) for the period from the 1st of January to the 1st of December 2004.

Specimens were collected in disposable sterile plastic containers which were delivered to the laboratory and examined within 2 hours of collection (no preservatives had been used) . Each specimen was examined macroscopically (visually) for the presence of blood, pus and mucus. After the macroscopic examination, each specimen was divided into 2 parts, as the first part was examined by the direct microscopic smear method by using physiological saline, same above procedure was performed once more by using lugols iodine solution instead of the saline to identify protozoal cyst or trophozoite of *E. histolytica* and *G. lamblia*. The Oocyst of *C. parvum* were shown as unstained colourless spherical to ovoidal structures, whereas the second part was prepared for staining methods (modified acid-fast staining technique [30] or sufranin-methylen blue [31]) .so each specimen was examined three times, once with normal saline, once with lugols iodine and once with staining technique.

Results

Out of the 600 diarrheic stool specimens (acute and persistent) that were examined, 208 were found infected with one or more of pathogenic intestinal protozoan parasites representing 34.6% of the total diarrheic stool specimens, whereas 292 cases (representing 65.4% of the total diarrheic stool samples) were found not infected with pathogenic intestinal protozoa and the reasons for diarrhea were considered due to other causes (bacterial, viral and others) (Table 1).

Table (2) shows the distribution of the single or mixed infections according to the protozoan types among the total pathogenic causes of diarrhea. These including 96 cases of *E. histolytica*, 66 cases of *G. lamblia* and 14 cases of *C. parvum* which represent 16%, 11% and 2.3% respectively out of the total 600 diarrheic stool specimens

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No significant differences (P= or< 0.0001) were noticed according to the sex variation (Table 3). Table (4) show the distribution of the single pathogenic protozoa infected cases according to the age groups in which the variation was highly significant (P > 0.01) between the age group of 0-20 months and the age group of 41-60 months. The highest prevalence rate was noticed with the age group 0-20 months followed by the age group 21-40 months, and, the lowest was in the age group of 41-60 months for the three pathogenic protozoa.

The non-pathogenic protozoan parasites like *Entamoeba coli*, *Chilomastix mesnili*, *Endolimax nana* and *Iodamoeba buttschli* had been detected abundantly in the collected specimens, however, they had been not counted in the results.

Table (1). The prevalence of pathogenic protozoan causes of diarrhea.

Total diarrheic examined cases	Total diarrheic cases due to pathogenic protozoa		Total diarrheic cases due to other causes*	
	№	%	№	%
600	208	34.7	392	65.3

* This including non-pathogenic protozoa, worms, bacteria viruses and others.

Table (2). Distribution of single or mixed infections according to the pathogenic protozoan types.

Total diarrheic examined cases	Total infected cases (single and mixed*) infection								Total diarrheic cases due to other causes	
	No						%			
	208						34.6			
600	Total single infected cases						Total mixed* infected cases		No	%
							No	%	392	65.3
							32	5.3		
	№		%							
	176		29.3							
	E. histolytica		*G. lamblia*		*C. parvum*					
	№	%	№	%	№	%				
	96	16	66	11	14	2.3				

- It means more than one parasite in each examined sample. In some cases we found a helminth's ova beside the protozoan.

Table 3. Distribution of infection according to the sex of children.

Parasite	Infected males (♂)		Infected females (♀)		Total	
	№	%	№	%	№	%
E. histolytica	52	8.7	44	7.3	96	16
G. lamblia	34	5.7	32	5.3	66	11
C. parvum	8	1.3	6	1	14	2.3
Total	94	15.7	82	13.6	176	29.3

Table 4. Distribution of single pathogenic protozoan infection according to the age groups

Protozoa	0-20 months		21-40 months		41-60 months		Total	
	№	%	№	%	№	%	№	%
E. histolytica	46	7.7	33	5.5	17	2.8	96	16
G. lamblia	28	4.7	24	4	14	2.3	66	11
C. parvum	8	1.3	4	0.7	2	0.3	14	2.3
Total	82	13.7	61	10.2	33	5.4	176	29.3

Notice: Every percentage of this table was calculated independently for each protozoon species.

Discussion

In this study, 208 cases out of 600 stool specimens (34.6%) were found infected with intestinal protozoan parasites. This prevalence rate correlates with the low hygienic, economic, social and educational standards of the research area, as well as the deterioration of the drainage system, water purification system and health services after the 2003 war in Iraq, and agrees with the finding of other studies [6]. The finding of mixed infection. of human intestinal tract with more than one parasite was common and had been reported by many other studies [9, 10 and 34]. In the current study, the results of mixed infection were not analyzed because of the difficulties in deciding which parasite was the actual cause of diarrhea. The prevalence of protozoan infection (single or mixed) according to the protozoan species, in which the highest rate of infection was with *E. histolytica* (16%) followed by *G. lamblia* (11%) and *C. parvum* (2.3%). These results are in complete agreement with other previous studies [9, 10 and 35]. *E. histolytica* is world wide in its distribution and is

very common in tropical areas which ranged from 1-50% [36]. Recently, *E. histolytica* has been reclassified into pathogenic (*E. histolytica* sensu strico) and non-pathogenic (*E. dispar*) species [36]. The classic stool ova and parasite examination is insensitive and can not differentiate *E. histolytica* from the non-pathogenic, but identical appearing parasite, *E. dispar*. This fact would make the prevalence rate of *E. histolytica* infection (16%) in this study is in doubt as the possibility of misdiagnosis between *E. histolytica* and *E. dispar* is very likely but, it is unavoidable because of the lack of other more sophisticated methods that can differentiate between the two very similar species of the genus *Entamoeba*.

The prevalence of *G. lamblia* in this study (11%) is similar to other study [38] in which the prevalence rate was 16% and a little bit higher than the range of prevalence rate in developed countries worldwide (6-8%) [39 and 40].

Several methods exist for detection of the parasite. Demonstration of trophozoites or cysts in the stool, called the ova and parasite (O&P) examination, is the traditional mean of diagnosis [41]. One stool sample will allow the detection of 60-80% of infection, 2 stool samples will allow the detection of 80-90%, and three stool samples will allow the detection of over 90% [42 and 43]. However in some instances, because of intermittent or low level of shedding, it is necessary to examine more than three stool samples. In this study, one stool samples for each patient was examined because of the field difficulties in the following-up process of patients to get more than one stool sample, thus, the prevalence results of this study of *G. lamblia* (and *E. histolytica*) may represent 60-80% of the real prevalence rate among the examined stool specimens. Other methods for the detection of *G. lamblia* , as well as *E. histolytica*, like ELISA, non-enzymatic immuno-assays or fluorescein –tagged monoclonal antibodies can be superior diagnostic method to the O&P examination [44, 45, 46 and 47].

C. parvum is a common enteric protozoan parasite. In this study, the prevalence rate of this protozoon was 2.3% of the total diarrheic stool specimens, and this finding is similar to other studies in Iraq [10] and Pakistan [48]. Studies of childhood infection in industrialized nations indicated that 3% of children with diarrhea were infected with *C. parvum* [28]. The prevalence rate of *C. parvum* in this study is also in consistency with other studies in other countries as those reported in Turkey; 3.5% [49] and Burkina Faso; 7.5% [50], but it was much lower than those reported in some African countries as Zambia; 18% [51] and Uganda; 25% [29]. The reasons for this variation in the prevalence of *C. parvum* are not clear, but it is possible that the application of new molecular techniques in some of the above mentioned studies could have contributed to the considerably higher detection rate.

In this study, there were no significant differences in the prevalence rate of all pathogenic protozoan parasites between both sexes. This result is in convenient with other studies [8, 9, 21 and 52].

Referring to the variation in results according to the age groups in this study, the prevalence rate of the 3 pathogenic protozoan parasites was significantly higher ($P>0.01$) for the 0-20 months age group when compared with the other two age groups (21-40 and 41-60 months). These results are in agreement with other studies [10, 53 and 54].

The high prevalence rate among the age group 0-20 months was expected since a high proportion of the children in this age acquire the infection for the first time [53]. The occurrence of *C. parvum* and other protozoan parasites in a large proportion of diarrheic children less than 3 years of age has been reported in many of the developing countries including those in South Africa [54] and sub-Saharan Africa [55]. Children are usually exposed to protozoan parasite within a few weeks after birth, and with maternal protection through breastfeeding (and the prenatal placenta transferred immunity), symptomatic infections sometimes are delayed until 6-24 months of age [29]. An overlapping between the shedding of the maternal protection and the emerging of the infant immunity is most likely to occur during the first year of life which makes the infection with parasitic diseases is very fluctuating. In the current study, the decreased prevalence rate with the progress of age in the other age groups (21-40 and 41-60 months) was also expected because of the developed immunity of the child, on one hand, and the recurrent exposure to the same protozoon, on the other hand, which will provide a relatively protecting immunity level for the child, in spite of the increasing risk factor of exposure to different types of parasites with different strain, which correlates with the age increasing of the child.

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