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Comparative Clinical Pathology

ISSN 1618-5641

Comp Clin Pathol
DOI 10.1007/s00580-013-1814-5

Springer

Comp Clin Pathol
DOI 10.1007/s00580-013-1814-5

ORIGINAL ARTICLE

A comparative study on enteric parasitic infections in immunocompetent and immunosuppressed children in Egypt

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Received: 6 May 2013 / Accepted: 11 September 2013

Abstract Diarrheal diseases are widespread all over the world, not only threaten human health but also greatly affect society and economy. Immune status may affect parasitic infections and the ability to combat such diseases. We aimed to investigate the prevalence of parasitic infections in immunosuppressed pediatric patients in comparison to immunocompetent ones with gastroenteritis. A total of 189 stool samples were collected from 100 immunocompetent diarrheic children at Cairo University Children's Hospitals and 89 children with cancer and diarrhea at the National Cancer Institute (NCI), Cairo University. All cases were subjected to history taking and clinical examination. Stool samples were examined microscopically and by ELISA for *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium* oocyts. Frequency and duration of both diarrhea and vomiting were significantly higher in immunosuppressed children ($P<0.01$). *E. histolytica*, *Giardia,* and *Cryptosporidium* were detected in 2 (1.1 %), 29 (15.3 %), and in 9 (4.8 %) samples, respectively, whereas 46 (24.3 %) cases were positive for other parasitic infections. Overall parasitic infections were positive in 86 stool samples (45.5 %): 41 belonging to immunocompetent patients (41 %) and 45 (50.6 %) to the immunosuppressed group though with no statistically important difference. Parasitic infections in Egyptian children with gastroenteritis are still high. No difference in prevalence of infections between immunosuppressed and immunocompetent children, yet clinical data were statistically higher in immunosuppressed patients ($P<0.01$). ELISA is as good as microscopy and is advantageous in its ability to differentiate between *E. histolytica* and *Entamoeba dispar*, thus can aid routine labs in detecting parasitic pathogens.

Keywords Immunocompetent · Immunosuppressed · Intestinal protozoa · ELISA · Microscopy

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Introduction

Parasitic diseases continue to be a major cause of morbidity and mortality. More than three billion people are infected worldwide, mainly in the developing countries. Children are easy prey to parasites. Poverty, illiteracy, poor hygiene, lack of access to potable water, poor public health infrastructure, hot and humid tropical climate are the usual factors associated with intestinal parasitic infections (Evering and Weiss 2006; Steketee 2003).

In many instances, the ability to successfully combat parasitic infections requires that the host mounts an effective inflammatory response against the parasite. Acquisition of infection,

Published online: 24 September 2013

clinical severity, and outcome of a parasitic disease often depend on innate and acquired host immunity (Evering and Weiss. 2006). Thus it can be anticipated that immunocompromised children are prone to various infections including parasitic infections with protracted diarrhea and/or chronic malabsorption (Lewthwaite et al. 2005; Mariam et al. 2008).

Entamoeba histolytica, *Giardia lamblia*, and *Cryptosporidium* are three of the most common diarrhea-causing parasitic protozoa. For many years, microscopic examination of stool samples has been considered to be the "gold standard" for diagnosis of such protozoal infections. Recently, more sensitive alternative methods (such as PCR, enzyme-linked immunosorbent assay, and direct fluorescent-antibody assay) have been introduced for diagnosis of parasitic infections (Verweij et al. 2004).

In the last two decades, genetic and subsequently immunological differences between what was known as pathogenic and nonpathogenic strains of *E. histolytica* have been elucidated. Consequently, the two strains were classified as two separate species, named *E. histolytica* and *Entamoeba dispar*, respectively (Amond and Clark 1993).

As the potentially invasive *E. histolytica* is morphologically indistinguishable from the noninvasive *E. dispar*, microscopy alone cannot certainly diagnose infection with *E. histolytica* (Haque et al. 1997; Ravdin. 1994). Although both cross-react immunologically, yet monoclonal antibodies are able to distinguish between them (Verweij et al. 2003).

Thus in this work, in addition to microscopic examination, an ELISA has been employed, for the detection of *E. histolytica*, *Giardia*, and *Cryptosporidium* antigens and, in case of entamoebiasis, to establish certain diagnosis of *E. histolytica*.

The objective of this study was primarily to compare between parasitic infections in both immunocompetent and immunosuppressed children and to correlate clinical presentations with the parasitic disease in both groups. It aimed also to compare ELISA to conventional methods in diagnosing *E. histolytica*, *Giardia*, and *Cryptosporidium*.

Patients and methods

Study design and subjects

A cross-sectional study was conducted on 189 pediatric inpatients complaining of diarrhea (with more than four bowel motions per day) and belonging to two groups: group I comprising 100 immunocompetent children with diarrhea hospitalized in Cairo University Children's Hospitals (Tertiary hospital) and group II including 89 immunodeficient diarrheic children with different malignancies hospitalized in the National Cancer Institute (NCI) (Tertiary hospital) for chemo- and/or radiotherapy.

Stool sampling and analysis

Stool samples were obtained from all cases over the period from November 2011 till October 2012. Detailed history was taken and patients' data were collected. Cases were also subjected to complete clinical examination. The study was approved by the Ethical Committee Board of the University and parental consents were obtained.

Stool samples were destined for routine microbiological cultures including fungal culture on Sabaroud dextrose agar in order to exclude *Candida* as stool colonizer which might cause false-positive results in staining.

After recording the macroscopic appearance of all stool samples, a portion of the stool was subjected to microscopic examination as both wet preparations and formalin-ethyl acetate sedimentation concentration method using the low (×10) and the high (×40) power lens.

The whole stool samples were then divided into two aliquots in capped tubes. One aliquot was stored at −20 °C to be used for ELISA [TECHLAB® Blacksburg, VA 24060, USA] for qualitative detection of *G. lamblia* antigen (PT5012), *Cryptosporidium* antigen (PT5014), and *E. histolytica* adhesion (T5017) rather than *Entamoeba dispar*. The used conjugate is a monoclonal antibody-peroxidase conjugate specific for *E. histolytica* adhesion molecule; thus, if the adhesion molecule is present in the specimen, it will bind to the conjugate, to be immobilized by the polyclonal antibody during the incubation phase. The ELISA was performed according to the manufacturer's instructions (Delialioglu et al. 2004; Zeehaida et al. 2008). The other aliquot was preserved by adding 10 % formalin, to be stored in tightly capped tubes at room temperature for staining with *Kinyoun carbol fuchsin stain* [Biostain Ready Reagents Ltd., Manchester, England: commercially available stain solutions] for detection for detection of *Cryptosporidium* oocysts.

Observations regarding the gross picture of stool samples, in addition to the findings obtained on microscopic examination of wet and stained preparations and the ELISA results were collected and summarized. Samples were considered positive for parasitic infections if positive findings were obtained in both, microscopic examination and ELISA.

Data were statistically described in terms of mean ± standard deviation [±SD], median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student *t* test for independent samples in comparing two groups when normally distributed and Mann Whitney *U* test for independent samples when not normally distributed. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency was less than 5. *P* values less than 0.05 were considered statistically significant. All statistical calculations were done using computer programs SPSS [Statistical Package

for the Social Science; SPSS Inc., Chicago, IL, USA] version 15 for Microsoft Windows.

Results

The present study was conducted on 189 Egyptian diarrheic children, 100 immunocompetents from Cairo University Children's Hospitals (group I), and 89 immunosuppressed children admitted to the National Cancer Institute, Cairo University, Egypt, and constituting group II.

The study group comprised 107 males (56.6 %) and 82 females (43.4 %), living in different urban and rural governments all over Egypt (Table 1). Out of the 186 stool samples included in the study, a total of 86 (45.5 %)were positive for parasitic infections, distributed as 41 samples within group I (41 % of the immunocompetent children) and 45 out of the immunosuppressed children, making up for 50.6 % of this group with no statistically important difference.

Of both groups, 2 (1.1 %) cases were positive for *E. histolytica*, 29 (15.3 %) for *Giardia*, 9 (4.8 %) for *Cryptosporidium*, and 46 (24.3 %) cases were positive for other parasitic infections. All the children under the study complained of abdominal pain while 75 (39.7 %) gave history of recurrent gastrointestinal infections and 106 (56.1 %) were dehydrated; all were statistically significant ($P<0.001$). Also, among the studied group, 105 (55 %) complained of vomiting and 146 (77.2 %) had fever and both were statistically non-significant.

The mean of: duration of diarrhea, number of motions per day, duration of vomiting, and number of bouts of vomiting per day in both immunocompetent and immunosuppressed patients is shown in Fig. 1. That all these clinical data were higher in immunosuppressed patients was of statistical significance ($P<0.01$).

Macroscopic appearance of the stool samples collected in the present study revealed that samples (44.4 %) had a watery consistency, 46 (24.3 %) revealed mucus, and 48 specimens (25.4 %) contained blood.

Microscopic examination of the stool samples of the 189 children included in the study revealed that 7 (3.7 %), 31 (16.4 %), and 9 (4.8 %) were positive for *E. histolytica /dispar*, *Giardia*, and *Cryptosporidium*, respectively. On the other hand, ELISA detected only 2 (1.1 %) and 34 (18 %) cases for *E. histolytica* and *Giardia*, respectively, while for *Cryptosporidium*, the results of microscopy after Kinyoun carbol fuchsin

Table 1 Distribution of residence among the studied groups

Residence	Group I (100)	Group II (89)	Total (189)
Great Cairo	44 (44 %)	38 (42.7 %)	82 (43.4 %)
Delta region	16 (16 %)	22 (24.7 %)	38 (20.1 %)
Upper Egypt	40 (40 %)	29 (32.6 %)	69 (36.5 %)

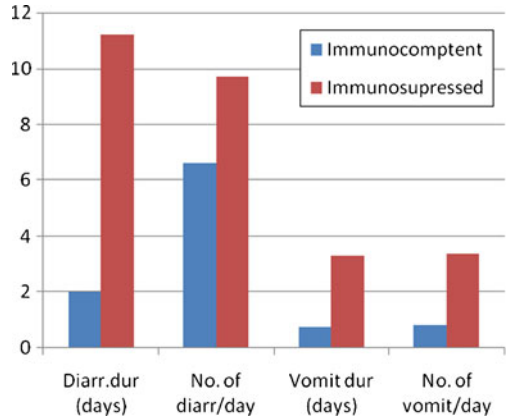

Fig. 1 The mean of: duration of diarrhea, frequency of diarrhea, duration of vomiting, and frequency of vomiting in both immunocompetent and immunosuppressed patients

stain and those of ELISA were identical (nine positive cases or 4.8 %). Detailed numbers of positive cases in immunocompetent and immunosuppressed children by both, microscopic examination and ELISA are summarized in Table 2. Comparatively, the final number of positive cases (i.e., positive in both tests) for each of the three protozoa searched for can be demonstrated in Fig. 2. Comparison between both tests was statistically significant ($P<0.01$).

Table 3 summarizes the clinical presentations of the final positive cases, in addition to the gross appearance of their stool specimens in terms of presence of blood or mucus.

Other microscopically detected organisms among the studied immunocompetent group included 14 positive samples for *Blastocystis*, 5 positive samples for *Entamoeba coli* cysts (including one sample positive for both, *Blastocystis* and *E. coli*). In addition, one sample was positive for *Endolimax nana* cysts, one for *Chilomastix mesnili* cysts, and one for *Hymenolepis nana* eggs.

On the other hand, in immunosuppressed cases 21 samples were positive for *Blastocystis* (23.4 vs 14 % in the immunocompetent children, which was statistically insignificance

Table 2 Positive number of cases by microscopic examination and ELISA and by both tests in immunocompetent and immunosuppressed children

		Mic[a]	ELISA	Both
E. histolytica	Immunocomp[b]	4 (4 %)	0 (0 %)	0 (0 %)
	Immunosup[c]	3 (3 %)	2 (2 %)	2 (2 %)
Giardia	Immunocomp[b]	18 (18 %)	19 (19 %)	16 (16 %)
	Immunosup[c]	13 (14 %)	14 (17 %)	13 (14 %)
Cryptosporidium	Immunocomp[b]	5 (5 %)	5 (5 %)	5 (5 %)
	Immunosup[c]	4 (5 %)	4 (5 %)	4 (5 %)

Numbers in this table are approximated to the nearest whole number

[a] Microscopic examination

[b] Immunocompetent

[c] Immunosuppressed

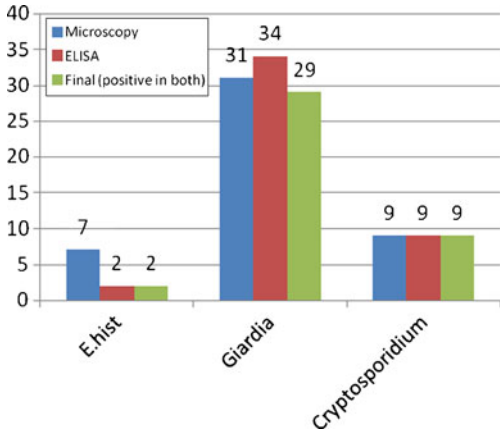

Fig. 2 Number of positive cases by microscopy, ELISA, and in both tests for each of *E. histolytica*, *Giardia*, and *Cryptosporidium*

$P=0.3$), 3 were positive for *E. coli* cysts and one was positive for *Iodamoeba butchlii* cysts.

The overall prevalence of parasitic infections in the studied group was 45.5 % (86 cases). After exclusion of protozoa considered to be commensals (five samples of *E. dispar*, eight samples of *E. coli*, one sample for each of *Endolimax nana*, *Chilomastix mesnili* and *I. butchlii*, the number of pathogenic parasitic infections were 70 (37 %), out of which 35 (18.5 %) were positive for *Blastocystis*.

Discussion

In the present study, the distribution of residence among the population of study was noticed to be high in Great Cairo 82 (43.4 %) compared to 69 (36.5 %) in Upper Egypt, while it was the lowest 38 (20.1 %) in the Lower Egypt (Delta region) (Table 1). This may be explained by low socioeconomic level of patients, living in Cairo, attending tertiary hospitals. It was reported in 2008 that almost 20 % of the Egyptian population is considered to live below the national poverty line (Access UNFPA 2008).

In this work, the number of parasitic infections in the study population (189 cases) was 86 (45.5 %), with 41 positive cases (41 %) among the immunocompetent patients and 45 positive cases (50.6 %) in immunosuppressed group, but this difference was not of statistical importance. However, the diarrhea and vomiting (regarding severity, frequency and duration) were statistically higher in immunosuppressed patients ($P<0.01$) (Fig. 1). Idris et al. (2010) reported a 57 % prevalence of parasitic infections in immunocompromised children with persistent and/or recurrent diarrhea.

In a study done by Abdel Hafeez et al. (2012) in El-Minya governorate, Egypt, using Giemsa and acid-fast staining for detection of protozoa in stool samples from 200 immunocompromised and 250 immunocompetent children, higher prevalence rates were recorded, amounting to 94 % in the immunosuppressed and 60 % among immunocompetent cases. These very high rates of infection in their results may be related to an epidemic or to use of different protocol (permanent stains) for detection of protozoa in addition to the different residence of their study population.

Another Egyptian study done in 2012 reported 47.6 % of the studied cases to be positive for giardiasis by microscopy while 61.9 % were positive by direct immunofluorescence assay (El-Nahas et al. 2013).

A recent study done in Tanzania (Speich et al. 2013) recorded closer results to that in the present study, reporting that 48.7 % of children in the study group had at least one of the protozoa: *Giardia intestinalis*, *E. histolytica*/*E. dispar*, and *Blastocystis hominis*.

Again, a study done by Menon et al. (1999) on parasitic infections in pediatric cancer patients reported close results to those of the present study, detecting parasitic infections in 42 % of studied cases, while another researcher showed lower prevalence rates, reporting parasitic infections in 33 % of the examined HIV-positive children in Thailand (Chokephaibulkit et al. 2001). Different epidemiological patterns in Egypt including behavioral, infrastructure, or environmental factors may be accused for these differences.

Regarding the recorded gastrointestinal manifestations and macroscopic picture of stool in enteric protozoa positive cases, abdominal pain was reported in (100 %) of cases (Table 3).

Table 3 Distribution of diarrhea related manifestations and macroscopic picture among positive cases

Protozoa	*E.hitolytica* ($n=2$)		*Giardia* ($n=29$)		*Crypt* ($n=9$)	
Characteristics	*n* (%)	*P* value	*n* (%)	*P* value	*n* (%)	*P* value
Abdominal pain	2 (100 %)	0.05[a]	29 (100 %)	0.001[a]	9 (100 %)	0.001[a]
Fever	0 (0 %)	0.5	22 (76 %)	0.8	8 (89 %)	0.9
Vomiting	2 (100 %)	0.05[a]	12 (41 %)	0.1	4 (44 %)	0.5
Dehydration	2 (100 %)	0.05[a]	11 (38 %)	0.04[a]	6 (67 %)	0.7
Recurrences	1 (50 %)	1.0	11 (38 %)	1.0	3 (33 %)	1.0
Mucus	2 (100 %)	0.05[a]	5 (17 %)	0.48	2 (22 %)	1.0
Blood	2 (100 %)	0.06	8 (28 %)	0.81	3 (33 %)	0.69

[a] Statistically significant

Recurrence of infection was strangely observed in 50, 38, and 33 % of entamoebiasis, giardiasis, and cryptospordiasis, respectively, which may be explained by incomplete treatment of patients for the recommended period of time after being discharged from the hospital due to lack of health awareness between their mothers.

E. histolytica cases showed significant higher percentages of vomiting, fever, and mucoid stool in comparison with *Giardia* and *Cryptosporidium* ($P=0.05$).

Studies in Bangladesh, India, and Australia have highlighted that *E. histolytica*/*dispar* infection among children is mostly associated with abdominal pain, distention, and fever and repeated infections; however, most of researchers did not differentiate between *E. histolytica* and *E. dispar* (Ali et al. 2003; Parija and Khairnar. 2005; Fotedar et al. 2007).

In the present study, fever was the commonest symptom (78 %) in giardiasis after abdominal pain (100 %), however it was statistically non-significant; while dehydration was recorded in (38 %) of *Giardia*-positive cases and was statistically significant ($P=0.04$).

Heresi et al. (2000) reported the occurrence of abdominal pain in 55–80 % of patients infected with *Giardia*. In another study performed at the laboratory of Hamadan, Iraq, the percentage recorded was (42.1 %) (Taherkhani et al. 2009).

In cryptosporidiosis, the commonest symptoms were abdominal pain, fever followed by dehydration; however, only abdominal pain was statistically significant ($P<0.001$).

Tzipori and Ward (2002) reported that cryptosporidiosis is a major factor in causation of dehydration, malnutrition, and effects on growth in children.

The commonest parasitic infection in this study was *Blastocystis* which was positive in 35 (18.5 %) with 14 (67 %) in immunocompetent and 21 (84 %) in immunosuppressed. However, the prevalence of *Blastocystis* could have been underestimated because the culture method is not performed as it is not the aim of the present study.

This was also reported by Idris et al. (2010) who recorded 55 % of the studied children to have *Blastocystis* based on in vitro culture. Another researcher showed in their study that *Blastocystis* was the commonest protozoal infection (Noureldin et al. 1999).

The results of the present study showed positive *E. histolytica*, *Giardia*, and *Cryptosporidium* by both microscopic examination and ELISA in 2 (1 %), 29 (15 %), and 9 (5 %) cases, respectively (Table 2). While all *E. histolytica* cases were in immunocompromised group, in giardiasis, 16 (16 %) cases were immunocompetent while 13 (14.6 %) were immunosuppressed. Regarding *Cryptosporidium* oocysts, five (5 %) cases were immunocompetent while four (4.5 %) cases were immunosuppressed.

On the contrary, Abdel-Hafeez et al. (2012) reported *Cryptosporidium parvum* to be the commonest parasite with a prevalence of 60.2 % in both immunosuppressed and immunocompetent children. This high prevalence may be related to the type of patients chosen in the research (immunosuppressed patients with chronic diarrhea, minimum of three loose stools per day lasting more than 2 weeks). In the same study, *G. lamblia* and *E. histolytica* prevalence was higher in immunocompetent children, being (17.6 %) and (24.6 %), respectively.

Another study reported closer prevalence for *E. histolytica*/*dispar* (0.74 %) to that of the present study (Akdemir and Helvaci. 2007).

In this study, microscopic examination revealed seven positive cases of *E. histolytica*/*dispar* (Table 2), while ELISA revealed only two positive cases of *E. histolytica*. This is because ELISA (*E. histolytica* II TECHLAB) is specific for detection of *E. histolytica* rather than not *E. dispar* owing to the monoclonal antibodies for specific epitopes in the galactose adhesion molecule of *E. histolytica* (Zeehaida et al. 2008).

Regarding *G. lamblia*, 31 (16 %) cases were positive by microscopy, while 34 (18 %) were positive by ELISA (*Giardia* II TECHLAB) and this was shown to be statistically significant ($P<0.01$).

The advantage of ELISA (*Giardia* II TECHLAB) over microscopic examination was supported by Nash et al. (1987) and Boone et al. (1999) who proved sensitivity and specificity of ELISA (*Giardia* II TECHLAB) as an alternative method for establishing the diagnosis as in cases of absence of the whole organism in stool. ELISA is also of benefit in examination of large number of specimens rapidly and objectively.

This was in accordance to Elgun and Koltas (2011), who concluded in their research that the results of copro-antigen ELISA indicate that it is a rapid, reliable, sensitive, and specific for routine diagnosis and may be useful for large-scale epidemiological studies of cryptosporidiosis.

For cryptosporidiosis, both microscopy and ELISA revealed positivity of nine (4.8 %) cases due to using of permanent stain (Kinyoun carbol fuchsin stain) in detection of *Crypyosporidium*.

Conclusions and recommendations

Enteric parasitic infections are a public health problem in Egypt. Awareness towards such a problem is still in need. Parasitic manifestations are more common in immunosuppressed patients. *Blastocystis* constituting the commonest protozoal infection, followed by *Giardia*, *Cryptosporidium*, and lastly, *E. histolytica*.

Clinical data were statistically higher in immunosuppressed patients. Abdominal pain and dehydration were observed in all cases, while vomiting and mucoid stool were statistically higher in *E. histolytica*. Recurrence of infection was observed

in 50, 38, and 33 % of entamoebiasis, giardiasis and cryptospordiasis, respectively, which may be explained by incomplete treatment of patients due to maternal unawareness.

ELISA was of great benefit over microscopy regarding its advantage of differentiating *E. histolytica* from *E. dispar*. Distinguishing between the two morphologically similar organisms is generally not widely applied except for research purposes, mostly using molecular techniques rather than the definitely simpler ELISA technique.

Regarding giardiasis, ELISA was more sensitive for detection of positive cases, while in cryptosporidiosis, both ELISA and microscopy gave similar results.

The present study recommends exerting more effort in the field of parasitic problems in Egypt. We also recommend the use of ELISA (TECHLAB) as a routine work in labs as it is a relatively easy and sensitive technique that does not depend on the experience of the microscopist and, the most important, is able to define disease as being due to actual infection with *E. histolytica*, distinguishing it from a mere coincident finding of the rather non-pathogenic *E. dispar*; the inability to differentiate them on microscopic basis being a factor in neglecting the search for the actual cause of clinical presentations of the patient.

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