Detection of Some Protozoan Parasites That Infect the Human Gastrointestinal Tract

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Abstract

Stool specimens were collected from patients who presented for various medical complaints in out patient Laboratories (The Central Health Laboratories / Baghdad). Every specimen was examined by Conventional microscopic examination (CME) and tested by IgG-ELISA kit.

Antibody against the *Entamoeba histolytica*, detected by ELISA, has the potential to become a valuable adjunct to blood diagnostics and make it more affective, although there is no replacement foe the microscopical examination because other potential pathogens could otherwise escape detection.

Our study was conducted in AL-Yarmok Hospital and the Central Health Laboratories in Baghdad to identify the prevalence of *E. histolytica* and *Giardia lamblia* among patients who attended these two clinics . General stool examination was carried out for each patient using direct method. The overall rates of protozoal infections with *E.histolytica* among all diarrheal cases were 40.6% ; 42.5% ;40% ;52.8% ; 47.5% ; 49.3 % and 46.1% in years between March 1999 to December 2006 respectively (except 2003), and for intection with *G.lamblia* were 22.3% ;20% ;15.6% ;13.6% ;23% ;17.2% and 23.4 % in years from March 1999 to December 2006 respectively (Except 2003).

The highest rate of infection with *E. histolytica* was 52.8% in 2002 and for *G.lamblia* was (23.4) % in 2006 . The highest rate of

infection (31 %) was among age group from birth to nine years old during the whole period of study.

There was no significant difference between the two sexes. In our study all samples of stool had pus cells.

E. histolytica was detected in 4(8%) stools by CME and 4(8 %) by G-ELISA.

Our results suggest that a single stool specimen examination will miss many pathogenic protozoan infections in symptomatic persons.

Introduction

Protozoa that infect the gastrointestinal tract include the deadly parasite *Entamoeba histolytica* and *Giardia lamblia* which are the most common causes of waterborne disease outbreaks. These species of parasites causing the chronic diarrhea especially in immunocompromised persons (1).

E. histolytica is an anaerobe parasite forming cysts which have four small nuclei and measure (10-15) μm in diameter. The cysts are strudy and resiste adverse environmental conditions. Some of the trophozoites of *E. histolytica* can invade the tissues of the large intestine and may erode them so extensively that they gain entrance into the blood stream (2). Thus, amoeba can reach all parts of the body. It is the causative agent of amoebiasis and amoebic dysentery .It inhibits the lumen and mucosa of the large intestine, predominantly the transverse colon and cecum (3). Members of all age groups and both sexes are infected. The risk of infection increases with inadequate sanitary conditions (4, 5). The infection with *Entamoeba histolytica* may lead to carcinoma of the colon, ulcerative colitis (6, 7).

Infection with *Entamoeba histolytica* may be identified by microscopical examination: stool examination; iron haematoxylin method and merthiolat iod formol concentration or serological examination like Enzyme Linked Immunosorbent Assay – ELISA (8, 9).

Dysentery and typhoid were becoming a real problem for children in Baghdad. To assist Iraqi children afflicted with these diseases. UNICEF is providing hospitals with intravenous fluids and oral rehydration salts, so children can recover (10). In April 2002, the AL-ALwiya pediatric hospital in Baghdad was received (595) diarrhea cases in children. Before and during the war, many people had shifted from Baghdad to rural areas, where they were drinking contaminated river water and polluted wells water (11)

The type of diarrhea can help in the diagnosis: If it is liquid and mixes readily with water rather than
Floating on top, and is not particularly foul smelling, the problem is likely something other than
giardiasis. Diarrhea that lasts less than a week, untreated, is probably not from giardiasis (12, 13).

Materials and Methods

(A) Population study (Group 1):

The study was conducted from March 1999 to December 2006 (Except 2003) including all attendants from places nearest to AL-Yarmok hospital and the Central Health Laboratories who were complaining of diarrhea. Nine hundred eighty fife (985) patients of both sexes and of all ages had been examined for detection of *Entamoeba histolytica* and *Giardia lamblia* in their stool.

Microscopic examination of stool samples:

The patients had given clean cups for the collection of stool samples, which were examined freshly by using lugal's iodine solution and normal saline solution.

Direct stool examination were done for detection of leucocytes (pus cells) and (Red blood cells) in all samples.

(B) Population study (Group 2) :

A study was conducted from January 2005 to December 2005 among all attendants from nearest places to the Central Health Laboratories who were complaining of diarrhea. Fifty patients of both sexes and of all ages had been examined for detection of antibodies against *Entamoeba hisolytica* antigen in their serum in addition to (10) patients as control.

Five milliliters of venous blood samples were drawn and stored at (4)°C for 15 min. The samples were centrifuged at speed of (3000) r.p.m. for (5) min. The serum was separated and placed in clean vials and kept frozen at (-20)°C until used. Fifty stool-specimens were collected from the same patients who presented for various medical complaints in out patient laboratories (The Central Health Laboratories/Baghdad) to detect the *E.histolytica* in each sample.

Blood examination by using *E. histolytica* IgG – ELISA kit:

A-IgG-ELISA kit (NovaTech Immunodiagnostic): it was used to detect the antibodies against the *E. histolytica.*

B- Sample dilution:

As instructed by the company processed (NovaTech Immunodiagnostic):

All samples should be diluted before assaying. Ten µl of sample and (1) µl of IgG sample diluent were disepened into tube to obtain a serum diluent and thoroughly mix with a vortex.

Positive and negative controls are ready to use and must not be diluted.

C- Test preparation:

As instructed by the company processed (NovaTech Immunodiagnostic):

1- (100) ml controls and diluted samples were dispensed into their respective wells. Wells were covered with the foils .

2-The plate was incubated for (1) hr at (37) °.

3- The foil was removed after incubation. All wells were washed three times with (300) µl of washing solution.

4- (100) µl of E. *histolytica* protein A conjugate was dispensed into wells and then were covered with the foil. The wells were incubated at room temperature for (30) minutes.

5-The step (3) was repeated. (100) µl TMB (Tetramethyl benzidine) substrate solution was dispensed into all wells. Then the wells were incubated at room temperature for (15) minutes.

(100) µl of stop solution (0.2mol/L sulphuric acid) was dispensed into each well. The absorbance of the specimen was measured at 450 nm within (30) min. after the addition of the stop solution .

Samples were considered positive if the absorbance value was equal or higher than over the determined cut – off. If the results of ELISA and microscopic examination were differ, ELISA results were compared with those obtained by Conventional Microscopic Examination (CME). The samples that had a positive result in CME were considered "true positive ".

Results and Discussion

It was found that the percentage of *Entamoeba histolytica* infection among (1519) patients of diarrhea were (40.6% ; 42.5% ; 40% ; 52.8% ; 47.5% ; 49.3% and 46.1%) while the percentages of *Giardia lamblia* were (22.3% ; 20% ; 15.6% ; 13.9% ; 23% ; 17.2% and 23.4%) in the years 1999 ,2000,2001,2002,2004,2005 and 2006 respectively Table (1).

The high rate of *E.histolytica* infectionis in similar to results of study done in Samaraa (56.7%) (14). *Giardia lamblia* was the leading parasite in Kerbala detected with a prevalence rate of (9.9%) followed by *E.histolytica* (3.5%) (15). Acute Giardiasis has been reported global prevalence of approximately (3o %) (16).

Regarding age Table (2) shows that the prevalence of protozoal diarrhea was the highest (31%) at age group of (0-9) years old followed by (30-39) years old age group (24.7%) respectively. These results were in agreement with the results of studies done by Kadir et al.(14), in Samaraa. The prevalence of protozoal diarrhea in Samarra was the highest (56.7%) in (0-9) years age group and (13%) in (30-39) years old age group .

By the direct wet smear, 7% (cyst) and 1.7% (vegetative) of *G.lamblia*; 5% (cyst) and 0.3% (vegetative) of *E.histolytica* were detected in stool samples of patients in Egypt (17).

Infections with *E.histolytica* occur during the past 2 decades , *Giardia* infection has become recognized as one of the most common cases of waterborne disease (found in both drinking and recreational water) in humans in the United States . Persons more likely to become infected include children and people who swallow water from contaminated sources (7).

In Our study, all samples of stool had pus cells. Some patients under study had many symptoms like bloody or mucoid diarrhea, fever and dehydration but many samples had not *E.histolytica* or *G.lamblia*. So, one stool specimen is not sufficient to detect protozoa in symptomatic patients. Most infections were detected in the first specimen or specimens submitted, but many were not detected until later examination (18).

Using microscopy , rates of detection for this parasite vary between 2 and 5% in industrialized countries (in USA) and between 20 and 30% in developing nations (19) .Examination of fresh stool for cysts and trophozoites and examination of a permanent stained smear are still the most widely used detection methods . Because the

parasites mechanically adhere to the intestinal mucosa by the ventral disk , a series of five to six stools may be examined with out recovering the organism (20). Procedures using the enzyme immunoassay (EIA) have been developed to detect *Giardia* spp. antigen in feces especially in children and 6 years old patieuts (21). The prevalence of intestinal parasitic infections was assessed (1993 through 1995) among two different groups of persons on the island of Bioko, Eqwatorial Guinea.

Parasitologic examinations were performed on stool specimens from a householdbased sample of 557 dwellers from the rural area of the island. The infection with *E.histolytica* was 14.9% while with*G.lamblia* was 7.2% (22).

Breast feeding particularly exclusive breast feeding is important for the prevention of presistent diarrhea. It reduces the incidence of acute diarrhea and continueing to breast-feed shortens individual episodes. (2 - 23)% of all diarrheal episodes became presistent; the incidence is highest during first and second years of life. Cases of fatility from presistent diarrhea are variable ranging from 23 to 62% of affected children under (5) year of age; half of them occur in the first year of life. WHO estimate that globally (35%) of all diarrheal deaths in children less (23, 24).

Regarding sex, Tabel (3) shows that there is no significant difference in protozoal diarrhea between the two sexes, being (35.4%) in males and (64.1%) in females. The result of another study done by Kadir et al., (14) who found that the prevalence of protozoal infection was higher in females (56.3%) in Samarra. No significant sex or age differences in the distribution of infection were detected in Karbala 1993 (15). *E. histolyica* infection was detected at least once in (80%) of the children who completed (4) years of observation in Bangladesh (25).

Regarding month distribution of protozoal diarrhea, its prevalence was (25.4%) in March, (17%) in April, (15%) in May, (11.4%) in June, (25.3%) in July, (8.2%) in August, (5.1%) in September, (3.1%) in October, (3.1%) in November and (9.6%) in December. These differences had no statical signifcance Table (4).

Regarding month distribution of protozoal diarrhea in Samarra, the prevalence was (59.5%) in March, (54%) in April, (56.1%) in May, (57.3%) in June, and (59.3%) in July (14). In Mexico City, there were (411) dealth from 3757 on 13 March 1997 to 8671 (902) dealth

on 23 March. Ghana has been reported in the upper east region (6364 cases, 497 deaths) (16).

Data on the prevalence of asymptomatic carriers of *E.histolytiea*, their likehood of progression to invasive disease, and the frequency of transmittion between urban and rural areas must be obtained.

In this study, we tested the sera of patients by ELISA kit (NOVATEC, immunodiagnostica, GmbH, Germany) that detects the antibodies against the *Entamoeba histolytica*. The results were compared with those of conventional microscopic examination. (50) specimens of stool were examined by CME and (50) speciemes of blood G-ELISA (in the same patients who took from them the stool samples), *E.histolytica* was detected in 4(8%) stools by CME and 4(8%) sera by G-ELISA Table (5). (46) stools were negative for *E.hisolytice* by both methods. (1) specimen was positive by G-ELISA and negative by microscopy, this sample was considered to be "false positive". There was (1) speciemen that was positive by microscopy and negative by G-ELISA "false negative". All of true positive samples were related to women.

Of the patients with intestinal protozoa, 34 (16.2%) had E.histolytica / dispar by stool examination of stained smears. By using ELISA for detection of *E.histolytica* adehsion in stool samples of (115) with diarrhiea only (18) had true *E.histolytica* infection. ELISA did not cross-reacted *E.coli*, *Giardia lamblia* and *Cryptosporidium*. So, ELISA for detection of *E.histolytica* adhesion in stool samples was more specific than microscopy detection (26).

The diagnosis of giardiasis and intestinal amoeboiesis is still primarily based on microscopical detection of the organisms in stool but it's time and labour are intensive and depend on the skill of an experienced microscopist (27). Because of the intermitted shedding of parasists it takes the microscopic examination of (3) consecutive stool-specimens to reach a sensitivity over (90%). Another difficulty in the microscopial diagnosis of intestinal amoebiasis is to distinguish the morphological identical non-pathogenic *Entamoeba* dispar from pathogenic *E.histolytica* (28). Given these difficulties the development of sensitive, cost- effective and rapid diagnostic methods is of most importance. So, the ELISAs for detection the specific antigens stool or antibodies against the *E-histolytica* and *Giardia lamblia* were developed.

The prevalence and distribution of *E.histolytica* in the Venda region (South Africa) were determined in stool samples collected from

public hosbitals and primary schools by ELISA. *E.hisolytica* was detected in 37/197 (18.8%). Age group most infected was 0-2 (33%). *E.histolytica* infections are common in the South Africa and are associated with diarrhea and intestinal inflammation (3).

Diagnosis of amebiasis is usually performed on a clinical basis alone in most endemic counteries having limited economic sources. But now in some counteries, like Turkey, United Kingdom and United States the researchers use the modren diagnostic tests for amebiasis.A total of (380) stool specimens were collected from Van and Harran University in Turkey and examined for *Entamoeba* by light microscopy (fresh, lugol, and trichrome staining) and stool antigen detection based enzyme linked immunosorbent assay (EIA). 13% (51/380) of stool specimens were found to be positive for *E.histolytica* by the EIA test, including 15% (14/91) of microscopy (+) stool specimens and 13% (37 / 289) of microscopy (-) stool specimens. 11.5% of *Giardia lamblia* was found in the same stool specimens (23).

The prevalence of *E.histolytica* detected by microscopic examination was (12.8) % rural Mexican community; serum IgG were detected using ELISA. 5.3% (1 / 19) of the serum samples were positive for IgG anti-amebic antibodies in patients with diarrhea in Mexico (29).

The prevalence of infection is as high as 50% in areas of central and South of America, Africa, and Asia in USA, amebic liver abscess is 7-12 times more common. In men than in women, although the sex distribution is equal in children and amebic colitis affects both sexes equally. The microscopic examination of a single stool specimen from patient with amebic colitis is only 33-50 % sensistive. Serum antiamebic antibody tests are an important adjunct to antigen detection particulary in cases of amebic liver abscess, in which the parasite is found less commonly in the stool. Tests for antibodies to amebae arc 90% sensitive for amebic live abscess and 70% sensitive for amebic colitis. A problem with serological tests is that the antibody presists for years after the initial infection; therefor, differentiating between current and previous infections may be difficult, especillay in individualls from endmic areas (30).

Recommendation:

We recommended to exam the blood samples with PCR to diagnose the parasites in the blood of patients, in addition to compare

the results of diagnosis of stool samples with ELISA tests to detect the *Giardia Lamblia.*

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Table (1): The prevalence of protozoal diarrhea in Baghdad city according to species of parasite

Year	Species of parasite	No. Examined	Positive cases	Trophozoite	Cyst	Mixed	Percentage%
1999	*E. histolytica*	251	102	26	61	15	40.6
	G. lamblia	251	56	14	36	6	22.3
2000	*E. histolytica*	200	85	16	40	29	42.5
	G. lamblia	200	40	4	16	20	20
2001	*E. histolytica*	225	90	20	51	19	40
	G. lamblia	225	35	8	26	1	15.6
2002	*E. histolytica*	180	95	21	42	32	52.8
	G. lamblia	180	25	12	6	7	13.9
2004	*E. histolytica*	200	95	28	65	2	47.5
	G. lamblia	200	46	16	26	4	23
2005	*E. histolytica*	203	100	25	70	5	49.3
	G. lamblia	203	35	6	20	9	17.2
2006	*E. histolytica*	260	120	25	71	24	46.1
	G. lamblia	260	61	12	36	13	23.4
	Total	1519	985	233	566	186	64.845
	Chi-sq= 0.979	d.f.=0.003		(P≤0.0001)			

Table (2): Age prevalence (in male and female) of protozoal diarrhea from March 1999 to December 2006 (except 2003) AL- Yarmok Hospital and Central Health Laboratories / Baghdad city

Age group	Year	Total positive cases	%
0-9	1999-2006	305	31
10-19	1999-2006	125	12.7
20-29	1999-2006	193	19.6
30-39	1999-2006	243	24.7
40-49	1999-2006	81	8.2
50-59	1999-2006	24	2.5
60-69	1999-2006	14	1.3
Total		985	100%
Chi-sq=4.35	d.f.=3.02	($p<0.0001$)	

Table (3): The prevalence of protozoal diarrhea in AL –Yarmok Hospital and Central Health Laboratories / Baghdad city / according to sex. (Except 2003)

Sex	Year	Positive cases	Percent %
Total no. of males examined	1999-2006	349	35.4
Total no. of females examined	1999-2006	636	64.6
Total		985	100%
Chi-sq=0.065	d.f.=1.09	($p\leq0.0001$)	

Table (4): The prevalence of rate protozoal diarrhea in Baghdad city / AL Yarmok hospital and CHL side according to month distribution in Years from March 1999 to December 2006 (except 2003).

Month (Year)	Positive Cases	%
March (1999-2006)	25	25.4
April (1999-2006)	168	17
May(1999-2006)	143	15
June(1999-2006)	112	11.4
July(1999-2006)	249	25.3
August(1999-2006)	81	8.2
September(1999-2006)	50	5.1
October (1999-2006)	31	3.1
November(1999-2006)	31	3.1
December (1999-2006)	95	9.6
Total (1999-2006)	985	123.2
Chi- sq=3.45	d.f.=0.398	(P<0.005)

Table (5): Percentage of infection with *E.histolytica* tested by microscopic examination and G- ELISA.

Parasite	Microscopical examination (N=50)			IgG-ELISA (N=50)		
	Positive	Negative	%	Positive	Negative	%
E. histolytica	4	46	4/50(8%)	4	46	4/50(8%)

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التحري عن بعض الطفيليات التي تصيب الجهاز المعوي المعدي للأنسان

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الخلاصة

جمعت 50 عينة براز من المرضى الوافدين الى مختبرات الصحة المركزي في بغداد، فحص كل أنموذج بوساطة الفحص المجهري.كما تم التحري عن الاجسام المضادة ضد الطفيلي *Entamoeba histolytica* بوساطة تقنية الادمصاص المناعي المرتبط بالانظيم IgG- ELISA لعينات الدم للمرضى المصابين بالطفيلي .

تمت دراستنا في مستشفى اليرموك التعليمي ومختبرات الصحة المركزي في بغداد لمعرفة تواجد طفيلي*Entamoeba histolytica* و *Giardia lamblia* ضمن المرضى الوافدين لهذين الموقعين . اجري فحص البراز العام لكل مريض باستعمال الطريقة المباشرة، اذ كانت نسبة الاصابة بطفيلي *Entamoeba histolytica* ضمن كل حالات الاسهال (40.6 ، 42.5 ، 40.6 ، 46.1 ، 49.3 ، 47.5 ، 52.8)% في السنوات من 1999 الى 2006 على التوالي (ما عدا 2003). اما طفيلي *Giardia lamblia* فكانت نسبة الاصابة (13.6 ، 15.6 ، 20 ، 22.3 ، 23 ، 17.2 ، 23.4)% في السنوات من 1999 الى 2006 على التوالي (ماعدا عام 2003) .

كانت اعلى نسبة اصابة بطفيلي *Entamoeba histolytica* (52.8 %) في عام 2002، وطفيلي *Giardia lamblia* (23.4 %) في عام 2006 واعلى نسبة اصابة ضمن الفئة العمرية من حديثي الولادة الى اطفال بعمر 9 سنوات، اذ كانت (31 %) في عام 1999 الى 2006 . لا توجد فوارق معنوية بين الجنسين . وكانت جميع نماذج البراز حاوية على خلايا قيحية . وشوهد طفيلي *Entamoeba histolytica* في عينات البراز التي فحصت، اذ كانت 4 (8 %) باستخدام المجهر (الطريقة المباشرة)، و 4 (8 %) بطريقة G-ELISA .استنتاجاتنا تشير الى ان فحص عينه واحدة من البراز سوف تخطي العديد التحليلات للاجابات الطفيلية للمرضى المصابين .