Acta Scientific MEDICAL SCIENCES (ISSN: 2582-0931)

Volume 7 Issue 5 May 2023

Research Article

Serological and Parasitological Detection of Amoebiasis in Immunocompromised Patients with Diarrhea

Nawfal Y Al-Dabbagh[1]* and Ma'ali Ibrahim Younis[2]

[1]*Al-Kitab University, College of Medical Technology, Kirkuk, Iraq*

[2]*Department of Microbiology, College of Medicine, University of Mosul, Iraq*

***Corresponding Author:** Nawfal Y Al-Dabbagh, Al-Kitab University, College of Medical Technology, Kirkuk, Iraq.

Received: February 28, 2023

Published: April 10, 2023

Abstract

Objectives: To detect the presence of *E. histolytica* trophozoites and/or cysts by microscopical examination of fresh stool specimens obtained from cases and control groups. To detect the presence of *E. histolytica* cysts by using Zinc sulphate concentration method among negative stool samples. To detect the presence of *E. histolytica* specific antigen in stool samples of cases and controls by using ELISA technique. To detect the presence of anti- *E. histolytica* antibodies of IgG class in the serum of case and control groups. To evaluate other parameters including C-reactive protein, haematological and biochemical tests on the serum samples of both case and control groups.

Subjects and Methods: This study was carried out at Ibn-Al-Atheer Hospital, Ibn Sina Hospital and Hazim Al-Hafith Teaching Hospital. In Mosul city. Sixty immunocompromised patients with diarrhoea were studied. The collection of samples was done in the period from November 2009 to July 2010. The 60 cases included both leukaemic patients on chemo-therapy and patients with chronic renal failure on dialysis. Stool samples were examined for *E. histolytica* trophozoites or cysts directly by using a microscope, and the detection of parasitic antigens was done by using the ELISA technique. Serum samples were collected for the detection of anti- *E. histolytica* antibodies of IgG type. Thirty (apparently healthy) individuals with matched age and sex and suffering from diarrhoea were included in the study as a control group.

Results: Among the sixty cases and thirty control, 11.7% and 13.3% respectively were found to have *E. histolytica* infection by ELISA stool antigen test. Direct examination of fresh stool samples by saline and iodine together with the concentration technique shows a higher rate of infection at 26.7% and 10% in case and control groups respectively.

Examination of serum by ELISA detects anti-*E-histolytica* antibodies of IgG type in higher frequency 20% of cases and 33.3% of controls than those detected to have stool antigen positive.

Regarding age and sex, the incidence of infection was found to be higher below the age of 10 years old with no significant difference between age groups and also no significant difference in the incidence of infection related to sex.

The C-reactive protein test together with other biochemical and haematological tests was done to evaluate the level of immunity in case and control groups and significant differences were found, where the level of both humoral and cellular immunity in cases was lower than in controls.

Conclusion: Diagnosis of *E. histolytica* infection by ELISA for stool antigen has high sensitivity and specificity and is very useful to avoid overdiagnosis and treatment.

Keywords: *Entamoeba histolytica*; Stool Antigen; IgG Antibody; ELISA; Immunocompromised Patients

Introduction

Infectious complications have become frequent causes of morbidity and mortality in cancer patients, often replacing the primary disease as the leading cause of death [1].

In past years, the frequency of infectious diseases has risen owing to the increased use of corticosteroids in chemotherapeutic regimens [2]. Chemotherapy other than corticosteroids appeared to predispose the patients towards getting parasitic infections more than any other form of anticancer treatment [3].

Immunodeficiency (or immune deficiency) is a state in which the immune system's ability to fight infectious disease is compromised or entirely absent; an immunocompromised person may be particularly vulnerable to opportunistic infections, in addition to normal infections that could affect everyone [4]. Patients suffering from various malignancies present with varying degrees of immunodeficiency caused by their treatments or due to the disease process itself [5].

Amoebiasis is an infection of human intestinal and extraintestinal organs by the protozoan parasite *Entamoeba histolytica*. Studies have confirmed the existence of two genetically distinct but morphologically indistinguishable species of *Entamoeba*: *E. histolytica and E. dispar* [6]. Of these two organisms, *E. histolytica* is the pathogenic and the etiologic agent of amoebic colitis and liver abscess, while the other, *E. dispar* is a non-pathogenic species and has never been associated with disease. Differential diagnosis between the two species is essential both for treatment decision and public health knowledge [7].

WHO has suggested that *E. histolytica* should be specifically identified and, if present, treatment is crucial; on the contrary if only *E. dispar* is identified, treatment is unnecessary [8].

Traditionally, the diagnosis of *E. histolytica* infection has relied upon microscopic examination of cysts, or trophozoites in fresh or fixed stool specimens. Antigen detection assays have proved to be very useful in the diagnosis of some parasitic infections, including *E. histolytica* and *E. dispar* [9].

Serological techniques have been employed for many years in the diagnosis of amoebiasis [10]. In endemic areas, as many as 50% of people may be carrying antibodies to *E. histolytica* due to prior infections, which may be largely asymptomatic. It has been stated that IgG antibodies develop acutely in >90% of patients after they recover from infection [11]. The prevalence of amoebiasis varies with the population of individuals affected, differing between countries and between areas with different socioeconomic conditions, Sometimes up to 50% of the population is affected in regions with poor sanitary conditions [12].

The aims of the present study are to:

- Detect the occurrence of *E. histolytica* trophozoites and cysts by examination of fresh stool specimens microscopically among case and control groups of different age groups.
- Detect the presence of *E. histolytica* in patients stool by using concentration technique (Zinc sulphate) among negative stool samples.
- Detect the presence of *E. histolytica* antigen in diarrhoeaic stool of case and control groups by using ELISA technique.
- Detect the presence of anti *E. histolytica* antibodies of IgG class in the serum samples of case and control groups.

Materials and Methods

Study population

This is a case control-study carried out during the period from November 2009 to July 2010. The present study was conducted on 60 patients who had depressed immunity because of receiving cytotoxic therapy and radiotherapy for more than 4 weeks or due to chronic renal failure and complaining of diarrhoea whether acute or as repeated episodes, watery or dysentery. The patients enrolled in the study were recruited from Ibn Al-Atheer Teaching Hospital, Al-Razi Teaching Hospital and Hazim Al-Hafith Teaching Hospital, all located in Mosul city.

This study was conducted among patients from all age groups where careful history was taken from each diarrhoeaic patient according to a questionnaire sheet. The control group included 30 apparently healthy immuno-competent individuals with no underlying disease, no previous past medical history, no relevant family history of clinical importance and suffering from recent mild diarrhoea. These subjects were collected from out patients.

Collection of stool specimens

Stools specimens were collected from patients and controls in a tightly covered wide mouth, disposable plastic containers. These were labeled with number, date and name of each subject.

Fecal samples were examined macroscopically and microscopically by the direct method using normal saline and lugol's iodine solution. A portion of each stool specimen was fixed in 10% formal saline to be further examined by concentration technique. For ELISA a one gram portion of fresh stools was stored quickly in a freezer at -20°C prior to analysis. The parasite detection was done by conventional microscopy on direct saline and iodine wet mount preparations or using the concentration technique and subsequent staining with Lugol's iodine solution [13,14].

Collection of serum samples

Five milliliters (ml) of venous blood were obtained from each patient and control subject. Blood was put in a plain tube and centrifuged for 1015 minutes at 2500 RPM for serum separation. The separated serum was put in new plain tube and kept frozen at – 20°C until use. The frozen sera were used later on for detection of IgG antibody for *E. histolytica* by enzyme-linked immunosorbent assay kit. (RIDASCREEN *E. histolytica* antibody detection).

Examination of stool specimens

Macroscopic examination

This was used for evaluating the consistency, colour of stool samples, and a presence or absence of pus, mucous and/or blood in the stool.

Microscopic examination

Was done to identify *E. histolytica* cyst and trophozoite in the stool samples, by using wet mounts and concentration technique (zinc sulphate), according to [13].

Stool antigen test (RIDASCREEN Entamoeba C1701)

This test is an enzyme immunoassay for the qualitative determination of *Entamoeba histolytica* antigen in stool samples. The test was used according to manufacturer's instructions.

Test principle

In the RIDASCREEN *Entamoeba* test, specific antibodies were used in a sandwich-type method. *Entamoeba*-specific antibodies against the antigens of *Entamoeba histolytica* were applied to the surface of the well in the microwell plate. A suspension of the stool sample to be tested and the controls were pipetted into the wells of the microwell plate. Next, antibodies conjugated with peroxidase against the antigens of *Entamoeba histolytica* were added and the plate was incubated at room temperature [19-24]. In the presence of *E. histolytica* antigens, sandwich complexes consisting of immobilized antibodies, *Entamoeba histolytica* antigens, and conjugated antibodies form in the sample. Unattached enzymelabelled antibodies were removed during the washing phase. After adding the substrate, the attached enzyme changes the colour of the previously colourless solution in the wells of the microwell plate to blue if the test is positive. On adding the stop reagent, the colour changes from blue to yellow. The extinction is proportional to the concentration of *Entamoeba histolytica* antigens present in the sample.

Anti-Entamoeba *histolytica* antibody detection (RIDASCREEN *E. histolytica* IgG k 1721)

For the qualitative screening of serum IgG antibodies to *Entamoeba histolytica* using an Enzyme Linked Immunosorbent Assay (ELISA) technique. The test was used according to manufactures instructions**.**

Principle of procedure

The micro test wells were coated with *E. histolytica* antigen. During the first incubation with the diluted patients' sera, any antibodies which are reactive with the antigen will be bound to the coated wells. After washing to remove the rest of the sample, the Enzyme Conjugate was added. If antibodies have been bound to the wells, the Enzyme Conjugate will then be bound to these antibodies. After another series of washes, a chromogen (tetramethylbenzidine or TMB) was added. If the Enzyme Conjugate is present, the peroxidase catalyzes a reaction that consumes the peroxide and turned the chromogen from clear to blue. The addition of the Stop Solution ended the reaction and turned the blue color to a bright yellow.

Interpretation of Results - ELISA Reader

- **Positive** - Absorbance reading greater than 0.4 OD units.
- **Negative** - Absorbance reading less than 0.4 OD units.

A positive OD reading indicated that the patient may be infected by *E. histolytica*.

A negative OD reading indicated that the patient had no detectable level of antibodies. This may be due to lack of infection or poor immune response by the patient.

Interpretation of results - visual

Compare results to the controls. A sample should be interpreted as positive if the degree of color is significant and obvious.

C-reactive protein (CRP).

Principle

Latex particles coated with goat anti-human CRP antibodies are agglutinated when mixed with samples containing CRP. The procedure was done according to [16]. The presence of agglutination indicated a level of CRP in the sample equal or > 6 mg/L. The lack of agglutination indicated a CRP level < 6 mg/L in the sample. The titer was expressed as the reciprocal of the highest dilution showing macroscopic agglutination.

General blood parameters

The haemoglobin concentration, total white blood cell count, and differential white blood cell count were done according to conventional methods [14].

Biochemical tests

Total protein analysis was done according to method described by [15].

Serum albumin analysis was done according to [16].

Statistical analysis

The statistical methods used for the analysis of the obtained data were as follows:

- Standard statistical methods were used to determine the mean, standard deviation (SD), number, and percentage.
- Unpaired t-test was used to compare the measured parameters between patients' cases and controls.
- Chi-square test was used for the relationship between patients and controls according to the results of stool antigen and serum IgG tests.
- Fisher Freeman Halton test was used to find the relationship between patients and controls according to C-reactive protein test results.

Statistical test results were considered significant at p = 0.05 or less [16].

Results

The results of stool antigen and anti-E. histolytica IgG antibody tests in cases and controls

The two tests were performed by ELISA technique. Seven out of 60 cases (11.7%) and four out of 30 controls (13.3%) were found to be positive by stool antigen assay, however, the difference was not significant (P = 0.530). The anti-*E. histolytica* IgG antibodies were found to be positive in 12 out of 60 cases (20.0%) and in 10 out of 30 controls (33.3%), no significant difference between cases and controls (P = 0.165) as shown in (Table 1).

Stool Ag	Stool Antigen				Serum IgG			
	Cases		Control		Cases		Control	
	No.	%	No.	%	No.	%	No.	%
Positive	7	11.7	4	13.3	12	20.0	10	33.3
Negative	53	88.3	26	86.7	48	80.0	20	66.7
Total	60	100	30	100	60	100	30	100
Statistical analysis (chisquare) test	P = 0.530 (N.S)				P = 0..165 (N.S)			

Table 1: The results of stool antigen and anti-E histolytica IgG antibody tests by ELISA in case and control.

Comparison between the results of microscopical examination of stool and stool antigen test

Comparison between the results of two techniques used in the diagnosis of amoebiasis are shown in (Table 2) and these include the microscopical examination of fresh stool samples which include (direct method + concentration technique to detect a small number of the parasite in the stool samples by zinc-sulphate flotation technique. A portion of all stool samples was kept frozen to be studied later by the ELISA technique to detect the parasite antigen

in the stool samples. The comparison between the two techniques are shown in (Table 2) where 5 cases and 1 control appear to be positive and 42 cases and 24 controls appear to be negative by both tests, while total positive by microscopical examination were 16/60 cases and 3/30 controls and by stool antigen test 7/60 cases and 4/30 controls appear to be positive with a significant difference in the results of both tests.

Results	Stool Microscopical Examination			Stool antigen (ELISA)		
	Cases No.	Control (Negative)	Total	Cases No.	Control (Negative)	Total
Positive	1	2	3	5	11	16
Negative	3	24	27	2	42	44
Total	4	26	31	7	53	61
Statistical analysis using (fisher exact test)	P = 0.002 (Significant)			P = 0.003 (Significant)		

Table 2: Comparison between the results of microscopical examination of stool and stool antigen test.

The relation of age and gender to the rate of infection

The infection was found mainly in children under (10) years of age and in adults (40) years of age and above, in both cases and controls with no significant difference in the results of stool antigen and anti *E. histolytica* IgG tests (Table 3 and 4).

	Cases		Controls	
Age/years	Ag	IgG	Ag	IgG
≤ 1-5	2	3	1	4
6-10	1	2	2	2
11-15	1	1	1	1
16-20	1	2	1	1
21-25	1	1	1	1
26-30	1	1	1	1
31-35	1	1	1	1
36-40	2	2	1	2
41 ≥ 45	2	2	1	1
Total	7	12	4	11
P-value * Chi-square test	0.35(NS)	0.60(NS)	0.13(NS)	0.33(NS)

Table 3: Relation of age to the results of stool antigen test and anti *E. histolytica* IgG antibody test.

Gender	Stool antigen				Serum IgG antibody test			
	Cases		Control		Cases		Control	
	No.	%	No.	%	No.	%	No.	%
Female	5	71.4	2	50.0	4	33.3	4	40.0
Male	2	28.6	2	50.0	8	66.7	6	60.0
	7		4		12		10	
Statistical analysis (chi square) test	0.257 (NS)		1.0 (NS)		0.248(NS)		0.527(NS)	

Table 4: The relation of gender to the results of stool Antigen and anti-*E. histolytica* IgG antibody tests.

Comparison of stool antigen test and anti-*E. histolytica* IgG antibody test results between cases and controls

In this study (11.7%) case of were detected to have stool Ag positive while (20%) of cases have serum IgG antibodies against *E. histolytica* parasite, where these antibodies. appear after 1 week of infection and last for years., while in the control group were healthy individuals with diarrhea, a positive stool Ag test was detected in 4(13.3%), while 10(33.3%) controls appeared to have serum IgG Ab positive. These results are shown in (Table 5).

Diagnostic tests	Cases	Controls	P-value
Stool Ag	7(11.71%)	4(13.3%)	0.53
IgG Ab	12(20%)	10(33.3%)	0.165

Table 5: Comparison of stool antigen test and anti *E. histolytica* IgG antibody test results between cases and controls.

C-reactive protein (CRP)

The results of the CRP for the cases and controls are demonstrated in (Table 6). According to the serial dilutions (1/2-1/64) of the serum, the patients and the controls were compared statistically using FisherFreeman Halton tests. The CRP serum levels of cases that represent immunocompromised patients with diarrhea were significantly higher than those of the controls (P < 0.001).

CRP (mg/L)	Cases		Controls	
	No.	%	No.	%
3	34	56.7	19	63.3
6	10	16.7	6	20
12	1	1.6	1	3.3
24	6	10.0	4	13.4
48	6	10.0	0	0.0
96	3	5.0	0	0.0
Total	60	100	30	100

Table 6: Distribution of C-reactive protein (CRP) test results in cases and controls.

P-value < 0.001.

Using Fisher Freeman Halton test.

The values of complete blood count in cases and controls

The results of the general investigations were compared between the cases and the controls using an unpaired t-test as shown in (Table 7). There were significant differences between the patients and the controls for the HB, total white blood cell count (W.B.C) & neutrophils count, with P- values of < 0.001, 0.002, and 0.014 respectively. However, there was no significant difference between the patients and controls for the lymphocyte count, with a P-value of 0.234.

Investigation	Mean ± SD		* P-value
	Cases	Controls	
Hb	9.36 ± 2.0	12.43 ± 1.3	< 0.001
Total W.B.C	4.14 ± 3.1	5.90 ± 1.6	0.002
% Neutrophiles	58.43 ± 26.3	69.27 ± 5.0	0.014
% Lymphocytes	27.69 ± 19.6	30.33 ± 4.5	0.234 (NS)

Table 7: The values of complete blood count in cases and controls.

* Unpaired t-test.

The values of different biochemical tests in case and control groups

The results of biochemical tests were done on serum samples of both cases and controls which include the total protein, albumin, and globulin with mean ± SD as shown in (Table 8). Statistically significant differences were found between cases and controls for total protein and globulin (0.001 and 0.012) respectively.

Biochemical tests	Cases (60)	Controls (30)	*p-value
	Mean ± SD	Mean ± SD	
Total protein	5.894 ± 0.208	7.242 ± 0.174	0.001(S)
Albumin	3.723 ± 0.207	4.071 ± 0.171	0.324(NS)
Globulin	2.229 ± 0.209	3.171 ± 0.155	0.012(S)

Table 8: The values of different biochemical tests in case and control groups.

* Unpaired t-test.

Comparison between the methods used for diagnosis of *E. histolytica* infection

The results of the techniques used for the detection of amoebiasis are shown in (Table 9). In case and control groups respectively (26.7%), (10.0%) were detected to have *E. histolytica*/*E. dispar* infection by microscope. By stool Ag test (11.7%), (13.3%) were diagnosed to have *E. histolytica* infection, and by serology (20%), (33.3%) were detected to have anti –*E. histolytica* IgG antibodies.

Discussion

The detection of stool antigen in case and control groups

The detection *of E. histolytica* antigen in stool samples is quite important to prove the presence of infection. Approximately ninety

Methods for diagnosis	Cases		Controls	
	No.	%	No.	%
Wet mount + concentration techniques	16	26.7	3	10.0
Stool Ag test	7	11.7	4	13.3
Serum IgG Ab	12	20.0	10	33.3

Table 9: Comparison between the methods used for diagnosis of *E. histolytica* infection.

percent of *Entamoeba* infections are asymptomatic [17,18]. Risk factors that are associated with increased disease severity and mortality include young age, pregnancy, malignancy, malnutrition, alcoholism and corticosteroid use [18,19]. *The E. histolytica* infection was defined as a positive test results for amoebic antigen in stool [20,21].

In the current study, the number and frequencies of cases who had a positive stool antigen by ELISA were 7(11.7%) of total cases (60), these results are lower than that detected by microscopical examination which cannot differentiate between *E. histolytica* and *E. dispar*. This is in agreement with the results reported by [21], in which thirty one out of 209(14%) enrolled children in that study had *E. histolytica*-associated diarrhoea. Moreover, Delialioglu (2008) had reported that from 272 stool samples with diarrhoea 24 (11.3%) of the samples were positive for *E. histolytica* by ELISA-specific antigen test, which is in agreement with the present study [22] showed that 12 samples from 88 (13.6%) were positive for *E. histolytica* by using ELISA antigen test, which is also in agreement with the present study.

A study done by Haque and Coworkers [23] showed that PCR and antigen detection had comparable sensitivities 87% and 85% respectively when performed directly on fresh stool specimens.

In the control group of the present study, only 4/30 (13.3%) were found to be positive by stool antigen test and this is in agreement with a study done by Braga., *et al.* [24] where 11*0 E. histolytica* infection out of 375 secac of mild diarrhoea (14.9%) found to have stool antigen positive by ELISA.

In the current study, no significant difference was found in the rate of infection between the case and control groups, however the low prevalence of this parasitic infection in cases may not reflect the actual picture, since the use of antibiotics both in prophylaxis and empirical treatment of infections is widespread in our country. Braga and Coworkers [24] reported that an antigen detection test is a sensitive and technically simple tool for detecting *E. histolytica* infection with a sensitivity and specificity that is comparable to PCR and isoenzyme analysis.

Comparison between the microscopical examination of stool and stool antigen test

The diagnosis of intestinal amoebiasis is still primarily based on microscopical detection of the parasite (trophozoite or cyst) in stool, but it is labour intensive and depends on the skill of an experienced microscopist [25].

It has been stated that the diagnosis via microscopical examination of a single stool specimen has a low sensitivity and may miss therefore up to 50-60% of *E. hisolytica* infections. This is due to the intermittent shedding of the parasite [26]. Low sensitivity of microscopical examination (53.8%) has been reported by Delialioglu., *et al.* 2004. Given these difficulties, the development of sensitive, cost-effective and rapid diagnostic methods is of almost importance.

The ELISA assay for the detection of a specific antigen in stool holds the potential to become an efficient diagnostic technique in the detection of E*. histolytica* [7].

By using both stool (wet mount + concentration technique) the parasite was detected in 16 cases (26.7%) and 3 controls (10.0%) and the results in cases were higher than those detected by stool antigen test due to the inability to differentiate *E. histolytica and E. dispar* by microscope. The diagnosis of amoebiasis by microscopic identification of cysts or trophozoites in the stool is time-consuming and requires expertise and does not distinguish pathogenic *E. histolytica* from nonpathogenic *E. dispar* [27].

Jayshree., *et al.* [1] reported that *E histolytica/E. dispar* were detected in 8.5% of patients with malignancy on anticancer therapy. Another study in Makkah Al-Mukarramah compared the results of microscopy and stool antigen test showed that 64.8% *E. histolytica/E. disapr* infection was detected by microscopy, then ELISA test showed *E. histolytica* in 4(2.6%) out of 101 cases (*E. histolytica/E. disapr*) by microscopy [12]. In the current study 7

out of 16 cases by microscopy were detected to have *E. histolytica* by stool antigen, which means that the remaining 9 cases were *E. dispar*. Given the small number of studies, the pathogenic potential of *E. dispar* still remains controversial [28].

It has been reported that microscopy is unable to differentiate *E. histolytica* from *E. disapr*, and can be confounded by false-positive results due to the misidentification of macrophages and nonpathogenic species of *Entamoeba.*

In a recent study conducted in Turkey, the positivity of *E. hisolytica* was 20.4% by microscopy and 29.5% with *E. histolytica* antigen detection by using ELISA test in adults and children patients with bloody stool [22]. PCR has not been used in the present study for reasons of availability and expenditure and is time-consuming. The antigen detection test is rapid and simple and does not require any special equipment. It is presently the only practical means for diagnosis of *E. histolytica* infection [23,29,30].

Relation of sex and age to the occurrence of E. histolytica infection

The occurrence of *E. histolytica* infection by stool antigen test and serology show no significant difference between male and female in cases (P = 0.257 and 0.248 respectively) and in control (P = 1.0 and 0.527 respectively). Also there was no significant difference with age. Since the number of positive individuals in various age groups under study was small, so the statistical comparison could not be logical.

These results are in agreement with the results reported by Braga [24] who showed that the most affected age group for *E. histolytica* was the 15 years old with no remarkable decrease with age and no significant difference in colonization rates between males and females.

Al-Harithi and Jamjoom [12] show no significant difference in the rate of infection in relation to gender. Delialioglu and Coworkers [22] reported that the frequency of *E. histolytica* infection in the different age groups has no significant difference and this is in accordance with the current study.

Serum IgG antibody test results in the cases and controls

Serological examination of both intestinal and extraintestinal amoebiasis continues to be characterized by low sensitivity and specificity [30]. Studies of serum IgG antibodies estimated that these antibodies were also found during asymptomatic *E. histolytica* infection [31].

In the current study anti-*E. histolytica* IgG antibody was present in 20% (12/60cases) and 33.3% (10/30 controls) and these results are higher than that found by stool antigen because IgG antibody can not differentiate old from new infection, and some strains may have limited invasiveness, therefore serology cannot be used alone for the diagnosis, especially in endemic areas, and can be used as an aiding tool with the other techniques. Regarding the control group the present study showed a high sero-prevalence 33.3% than that detected by Braga., *et al.* [32] who reported that 25% (22/88) had anti-GalNac lectin antibodies.

The relation between the results of antigen test and serum IgG antibody in the current study showed a discrepancy in the result this might be due to that stool antigen detecting the new infection, while serum IgG detect both new and old infection and it might be due to the high levels of residual positive antiamoebic IgG antibodies in areas of endemicity, as antibodies were demonstrated to persist for many years without any sign of reinfection. These results are correlated with the results reported by Braga., *et al.* [32] in a study done in Brazil that showed that seropositivity rates were not statistically significantly different between individuals colonized or not colonized by *E. histolytica*. The lower frequency of IgG antibody in the cases (20%) than in controls (33.3%) in the current study were explained by doing two tests for evaluation of the level of innate and cellular immunity by using C-reactive protein test (CRP) test, hemoglobin (Hb) and white blood cell count (W.B.C) total and differential respectively. Stool and serum antigen detection assays are sensitive, specific (differentiating between strains), easy to perform, and can potentially diagnose early infection [33]. These assays use monoclonal antibodies to bind to epitopes found on *E. histolytica* which are not present on other non-pathogenic strains [33].

The level of anti-amoebic IgG antibodies remains elevated in the serum for years after infection [30,34]. However, it limits the usefulness of serological methods for distinguishing new infections from prevailing antibodies and makes the utilization of serological testing in endemic regions complicated. In these regions, seroprevalence rates (indicating the frequency of prior

amoebic infections) can be as high as 50% and a positive titer is very common in the absence of symptoms [34]. Unlike *E. dispar* infection by *E. histolytica* results in the development of antibodies which can be detected within 5-7 days from acute infection [35]. Combining serological testing with antigen detection is currently the best diagnostic approach [17]. Using combined technique will increase the specificity and sensitivity in the diagnosis of E. histolytica infection. Further, this method allows clinicians to distinguish acute infection from chronic or previously treated infection.

C-reactive protein (CRP) serum levels in amoebiasis

The serum levels of CRP in different titers were significantly higher in patients than in controls ($P < 0.001$). This result demonstrates the value of CRP in predicting the presence of amoebiasis and it may indicate a poor prognosis.

CRP is regarded as one of the acute phase proteins, which are components of the innate immune responses that increase after infections, trauma, burns, tissue infarction, inflammatory processes, and tumors [36,37].

The value of complete blood count in cases and controls

The cases were found to be anemic (P-value for Hb < 0.001), and leucopenia ($P = 0.002$) with low neutrophil count ($P = 0.014$). All these investigations indicate a depressed immune response in the cases compared to the control group. This anemia and leucopenia might be due to primary disease or to chemotherapy and bone marrow suppression. The lower percentage of IgG antibodies in cases than controls in the present study was also explained by doing a test for the detection of total protein level, albumin, and globulin in the serum of the cases and the controls, where the antibodies in the circulation are composed mainly of a globulin part which is essential in the synthesis of antibodies in the circulation, and the globulin and total protein appear to be significantly low in cases of the present study in comparison to control group (P-value = 0.012, 0.001 respectively). This hypoproteinaemia and hypo globulinaemia might be due to the effect of the primary disease in our cases (leukemia, lymphomas, or chronic renal failure) or due to drugs (corticosteroid, cytotoxics, or others) used in their treatment regimens, that affect the liver and renal function [38].

Conclusion

The Conclusion of this work shows that the detection of *E. histoytica* antigen in stool by ELISA has high sensitivity and specificity and is very useful in the diagnosis of *E. histolytica* infection in order to avoid overdiagnosis and treatment. Also, serology by ELISA detection of antibodies can be used as an aiding tool together with other techniques and cannot be used alone in the diagnosis, especially in endemic areas and depending on the invasiveness of the parasite strains. Finally, *E. histolytica* is not a significant cause of diarrhoea in immunocompromised patients, and it affects them at a similar rate as in immunocompetent individuals.

Further studies using highly purified & specific amoebic antigens from xenic *E. histolytica* cultures are necessary for determining any superiority of these antigens in the diagnosis of the disease. Efforts should be made to employ the technique of polymerase chain reaction (PCR) in the differentiation of *E. histolytica* and *E. dispar by* using faeces or amoebic culture. The ELISA for specific stool antigens is recommended for a more effective diagnosis of *E. histolytica.*

Bibliography

1. Jayshree S., *et al*. "Intestinal Parasitic Infections in Patients with Malignancy". *Journal of Diarrhoeal Diseases Research* 14 (1996): 44-45.

2. Abaza SM., *et al*. "Intestinal opportunistic parasites among different groups of immunocompromised hosts". *Journal of the Egyptian Society of Parasitology* 25 (1995): 713-727.

3. Pizzo PA. "Infectious complications in the immuno-compromised host". *Hematology/Oncology Clinics of North America* 7.4 (1993).

4. Rosen FS., *et al*. "The primary immunodeficiencies". *The New England Journal of Medicine* 333.7 (1995): 431-440.

5. Rolston KV and Bodey GP. "Infections in patients with cancer". In: Cancer medicine. 4th ed. Baltimore (1997).

6. Stanley SL. "Amoebiasis". *Lancet* 361 (2003): 1025-1034.

7. Haque R., *et al*. "Rapid diagnosis of Entamoeba infection by using Entamoeba and Entamoeba histolytica stool antigen detection kits". *Journal of Clinical Microbiology* 33 (1995): 2558-2561.

8. WHO. Amoebiasis. W.H.O. Weekly Epidemiological Record 72 (1997): 97-99.

9. Tanyuksel M and Petri A. "Laboratory diagnosis of amebiasis". *Clinical Microbiology Reviews* 16.4 (2003): 713-729.

10. Fotedar R., *et al*. "Laboratory diagnostic techniques for Entamoeba species". *Clinical Microbiology Reviews* 20 (2007): 511-532.

11. Ravdin JI., *et al*. "Association of serum antibodies to adherence lectin with invasive Amebiasis and asymptomatic infection with pathogenic Entamoeba histolytica". *The Journal of Infectious Diseases* 162.3 (1990): 768-772.

12. Al-Harthi A and Jamjoom B. "Diagnosis and Differentiation of Entamoeba Infection in Makkah Al Mukarramah Using Microscopy and Stool Antigen Detection Kits". *World Journal of Medical Sciences* 2.1 (2007): 15-20.

13. Paniker J. "Textbook of medical parasitology". (2nd ed.) Jaypee Brothers medical publishers (2002): 15-27.

14. Daice and Lewis. "Practical haematology". Churchill living stone. Edinburgh, London, Melbourne and New York. 1984, Chapter (11) pp.179182, (25): 420-422.

15. Tietz N.W. "Text book of clinical chemistry". 3th ed. C.A. Burtis, E. R. Ashwood, W. B. Saunders (1999): 477-530.

16. Bettyr RK. "Essentials of medical statistics". Blackwell scientific publications (1988): 41-45 and 46-56.

17. A Cheepsattayakorn and R Cheepsattayakorn. "Parasitic pneumonia and lung involvement". *BioMed Research International* (2014).

18. S L Stanley Jr. "Amoebiasis". *The Lancet* 361.9362 (2003): 1025-1034.

19. T Wuerz., *et al*. "A Review of Amoebic Liver Abscess for Clinicians in a Nonendemic Setting". *Canadian Journal of Gastroenterology and Hepatology* 26.10 (2012): 729-733.

20. Haque R., *et al*. "*E. histolytica* infection in children and protection from subsequent amebiasis". *Journal of Infectious Diseases and Immunity* 74.2 (2006): 904-909.

21. Haque R., *et al*. "Correlation of interferon production by peripheral blood mononuclear cells with childhood malnutrition and susceptibility to amebiasis". *American Journal of Tropical Medicine and Hygiene* 76.2 (2007): 340-344.

22. Delialioglu N., *et al*. "Detection of *E. histolytica* antigen in stool samples in Mersin, Turkey". *Journal of Parasitology* 94.2 (2008): 530532.

23. Haque R., *et al*. "Comparison of PCR, isoenzyme analysis, and antigen detection for diagnosis of infection". *Journal of Clinical Microbiology* 36 (1998): 449-452.

24. Braga L., *et al*. "*Entamoeba histolytica* and *Entamoeba dispar* infections as detected by monoclonal antibody in an urban slum in Fortaleza, Northeastern Brazil". *Revista da Sociedade Brasileira de Medicina Tropical* 34 (2001): 467-471.

25. Thompson R., *et al*. "Giardia and Giardiasis". *Advances in Parasitology* 32 (1993): 71-160.

26. Ravdin JI. "Amebiasis". *Clinical Infectious Diseases* 20 (1995): 1453-1464.

27. Haque R., *et al*. "*Entamoeba histolytica* and *Entamoeba dispar* infection in children in Bangladesh". *The Journal of Infectious Diseases* 175 (1997): 734-736.

28. Kantor M., *et al*. "Entamoeba Histolytica: Updates in Clinical Manifestation, Pathogenesis, and Vaccine Development". *Canadian Journal of Gastroenterology and Hepatology* (2018): 6.

29. Delialioglu N., *et al*. "Detection of Entamoeba histolytica/ Entamoeba dispar in stool specimens by using enzyme-linked ımmunosorbent assays". *Memórias do Instituto Oswaldo Cruz* 99 (2004): 769-772.

30. Guillen M., *et al*. "Seroprevalence of anti-E.histolytica antibodies by IHA and ELISA assays in blood donors from puebla, Mexico". *Archives of Medical Research* 31 (2000): 53-54.

31. Haque R., *et al*. "Prevalence and immune response to Entamoeba histolytica infection in preschool children in Bangladesh". *American Journal of Tropical Medicine and Hygiene* 60 (1999): 1031-1034.

32. Braga L., *et al*. "Seropositivity for and intestinal colonization with *Entamoeba histolytica* and *Entamoeba dispar* in individuals in northeastern". *Brazil Journal of Clinical Microbiology* 36 (1998): 3044-3045.

33. R Haque., *et al*. "Diagnosis of pathogenic Entamoeba histolytica infection using a stool ELISA based on monoclonal antibodies to the galactose-specific adhesin". *The Journal of Infectious Diseases* 167.1 (1993): 247-249.

34. Khan P., *et al*. "Review of 100 cases of intestinal amoebiasis in SWAT". *Journal of Postgraduate Medical Institute* 19.2 (2005): 216-219.

35. KM Peterson., *et al*. "Enteric Amebiasis". in Tropical Infectious Diseases: Principles, Pathogens and Practice, R. Guerrant, D. H. Walker, and P. F. Weller, Eds., p. 614, Saunders Elsevier, Philadelphia (2011).

36. Gabby C and Kushner A. "Acute phase proteins and other systemic response to inflammation". *New England Journal of Medicine* 340 (1999): 448-454.

37. Coussens LM and Werb Z. "Inflammation and cancer". *Journal of Nature* 420 (2002): 860-867.

38. Hoffbrand A., *et al*. "Postgraduate haematology". (4th ed) London. ARNOLD (2001): 257-260.