

**Detection of *Entamoeba histolytica* antigen in stool specimens by
using ELISA in Basrah province**

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Abstract

In the present study, we have been used an Enzyme Linked Immunosorbent Assay (ELISA) for detection of *E. histolytica* \ *E. dispar* and comparing it with microscopic examination .Sixty five samples were examined by iodine staining was positive in 35 (53.6 %) then these iodine positive samples examined by ELISA a lotof which 33 (94.2 %) were positive in the mean time 4 (13.33 %) were positive in ELISA although that they were negative in microscopic exam.It can be concluded that microscopical examination has low sensitivity and specificity in comparision with ELISA.

Key word: *E. histolytica*, ELISA, Basrah

Introduction

Amoebiasis is an infection of the large intestine caused by the parasitic protozoan *E.histolytica* [1]. It is well known that annually 500 million individuals are infected world wide, resulting in 100000 deaths [2].However , the true distribution of the disease is not clear in Iraq especially in Basrah province.

E.histolytica actually indistinguishable species, *E.histolytica* can cause invasive intestinal and extra intestinal disease ,while *E.dispar* and other species can not [3].Thus the correct identification of this parasite is very important since *E.histolytica* is only species with in the genus *Entamoeba* required treatment.

Diagnosis of *E.histolytica* is usually based on microscopic examination of protozoan morphology [4].However, it reach about 60 % sensitivity and can give false positivity due to misidentification of nonpathogenic *Entamoeba* species [5].

Methods for antigen detection of *E.histolytica* by using ELISA in stool and polymerase chain reaction (PCR) have evaluated as diagnostic tools [2].

The present study have been designated to detect *E.histolytica* by using ELISA.

Materials and Methods

Stool specimens were collected from patients with blood and \ or mucus diarrhea at December 2008 and April 2009 at the Al- Fayha hospital in Basrah province.

A total of 65 stool specimens were iodine stained and examind by microscopy according to [3]. *E. histolytica* antigen (REF6004,Ludwig – Erhared – Ring ,15827 Dahlewitz,GERMANY).Detection was performed by ELISA according to manufacturers instruction. Briefly , 96-well microtiter ELISA plates were coated with polyclonal antibodies to *E.histolytica* peptide 1 (rabbit).Stool specimens were diluted 1: 10 in dilutents provided with the kit. Assay microtiter wells were incubated with 100 µl diluted samples and two drops of negative control and two drops of positive control , seal plates incubated at 30 min at room temperature (20 – 25 C °).The contents of the well strips were decant and were washed five time by using 300 µl of wash solution.After that two drops of conjugate added and the strip was incubated at room temperature .The contents decant , two drops of substrate solution was added and the strip was incubated at room temperature in the dark at 10 min.Following the incubation ,the reaction was stopped by adding two drops of stop solution to each well and mix gently , the absorbance was measured at 450 nm. The assay run was correct if the mean OD of the negative control is ≤ 0.2 or the mean of the positive control is ≥ 1 .

Results

Out of 65 stool samples examind microscopically 35(53.8%) were infected with *E.histolytica* and *E.dispar* complex parasite. The examination of the 35 positive samples by using ELISA test revealed that 33 (94.2 %) were positive for *E.histolytica* while after examing the rest 30 negative samples were given 4 (13.33%) (Table 1).

Evaluation of the sensitivity and specify of microscopic exam and ELISA test revealed that 24.6%,24.6%,94.2% and 100% respectively.

Comparison of characteristic stool samples by using microscopical and ELISA revealed that 5 (14.4 %) contained blood , 2(5.7%) contained blood and mucus , 4(11.4%) contained mucus and 3(8.5% %) were watery (Table 2).

Table (1) Comparison of samples by microscopical examination and ELISA results

Direct smear positive	ELISA positive	ELISA negative	Total
	33	2	35
Direct smear positive	4	26	30
Total	37	28	65

Table (2) Comparison of characteristic stool samples by microscopical and ELISA examination

characteristic	No. of samples	Microscopical exam		ELISA test	
		positive	negative	positive	Negative
Blood	5	5	-	5	-
Blood mucoid	2	2	-	2	-
Mucoid	4	4	-	4	-
semisolid	43	21	22	24	19
Watery	3	3	-	-	3
Pus cell	2	-	2	2	-
Solid	6		6		6
Total	65	35	30	37	19

Discussion

The recognition of *E.histolytica* and *E.dispar* as different species has provided important insights into the epidemic behavior of amoebiasis in the world [6] and had important epidemiological and clinical implications [7].

Antigen detection test are reported to be more sensitive and specific than direct microscopic examination [1] , Detection of antigen in the stool and serum is revolution in the diagnosis of *E.histolytica* due to the easy of use,high sensitivity and specificity of available test[8].Present study represent the first trying in Basrah province to specific identification of *E.histolytica* by using ELISA test.

In the present study microscopy had been 24.6 % sensitivity and specificity respectively, which were similar to that reported by [4] when they mentioned that sensitivity ranged between 20% to 60% and 10% to 50% specificity ,and the variation in the range of sensitivity and specificity may be due to effects of several factors which includes: lack of well trained microscopists ,delayed delivery to the laboratory and difficulty in differentiation between nonmotile trophozoites and polymorphonuclear leukocytes[9].

ELISA test has 94.2%,100% sensitivity and specificity respectively and this agreement with[1]that they found the sensitivity of this method was 95% [10].[11, 12] and [13], found that sensitivity and specificity were 87%,84%,83% and 92.5% respectively and this

different may be due to they using of monoclonal antibody for detection of antigen in stool while in the present study polyclonal antibody have been used.

It is well known that laboratory examination plays a major role in the diagnosis of intestinal amoebiasis, as well as the history of patient.

Although direct microscopic examination is unexpensive compared to antigen detection test, the subjectivity of the test ,the requirement for experienced microscopists due to the difficulty in differentiating the parasites from leucocytes and other intestinal parasites and its inability to differentiate pathogen species from nonpathogenic forms limits its reliability [14].The PCR technique is time consuming , expensive and therefore not well suited for use in developing countries where amoebiasis is prevalent [1].The antigen detection test has been shown to be suitable for the diagnosis amoebiasis in endemic areas [15]due to the test is rapid and simple and dose not require any special equipment, is presently the only practical means for diagnosis of *E.histolytica* infection [1].

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الكشف عن مستضد الاميبا الحالة للنسيج *Entamoeba histolytica* في نماذج الغائط باستعمال اختبار ELISA في محافظة البصرة

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الخلاصة

تم في الدراسة الحالية استعمال فحص الامتصاصية المناعية المرتبطة بالانزيم (ELISA) في الكشف عن الاصابة بطفيلي الاميبا الحالة للنسيج *E. histolytica* ومقارنته مع الفحص المجهرى للغائط (بطريقة المسحة المباشرة).

اشارت نتائج الدراسة الحالية الى كشف اصابة ٣٥ (٥٣,٦ %) من اصل ٦٥ عينة غائط تم فحصها مجهريا باستعمال صبغة اليود ، وعند استعمال اختبار ELISA كشفت اصابة ٣٣ (٩٤,٢ %) بالطفيلي فضلا عن كشف اصابة ٤ عينات (١٣,٣٣ %) من العينات التي كانت سالبة عند الفحص المجهرى .تم الاستنتاج بعد هذه الدراسة ان حساسية وخصوصية الفحص المجهرى اقل من حساسية وخصوصية اختبار .ELISA