

EVALUATION OF ANTIGEN-BASED ENZYME IMMUNO-ASSAY IN REFERENCE TO DIRECT MICROSCOPY FOR THE DIAGNOSIS OF ENTAMOEBIA HISTOLYTICA IN STOOL SAMPLES ⁺

Huda M. Jawad *

Ilham A. Majeed **

Nada M. Al-Basheer ***

Abstract:

In the present study, we have been used TechLab *Entamoeba histolytica* II Enzyme Linked Immune Sorbent Assay (ELISA) test for detection of *Entamoeba histolytica* and comparing it with microscopic examination.

A total of 80 stool samples were collected from patients with intestinal amoebae, including 47 males and 33 females, the age between (1-60) years. The stool samples were examined by direct microscopy ,then we investigated *E. histolytica* specific adhesin antigen in stool samples using ELISA(TechLab *E. histolytica* II test , United States of America USA). Out of 80 stool samples examined microscopically 65 were infected with intestinal amoebae. The examination of the 65 (81.3%) positive samples by using TechLab *E. histolytica* II ELISA test revealed that 13 (16.3%) were positive for *E. histolytica* while 2 (2.5 %) positive result using TechLab *E. histolytica* II ELISA kit was detected among the15 (18.8 %) microscopically negative samples. The study shows the majority of intestinal protozoa occur in males 36 (45.0%) and females 29 (36.3%), as well in *E. histolytica* high percentage were record in males 9 (11.3%) and in females 6 (7.5%), the results shows the highest percentage of intestinal protozoa as well *E. histolytica* at age group (30-39) (22.5%) (7.5%) respectively.

Conclusions:

- 1- Direct microscopy is of low specificity of diagnosis.
- 2- The detection of *Entamoeba histolytica* antigen in stool samples by using TechLab *E. histolytica* II ELISA is more specific in diagnosis of *E. histolytica* thus diagnosis of amoebiasis.

التحري عن مستند الأميبا الحالة للنسيج في عينات الخروج باستخدام تقنية TechLab
Entamoeba histolytica II ELISA

ندى محمد البشير

إلهام عبد الواحد مجيد

هدى محمد جواد

المستخلص

في هذه الدراسة تم استعمال فحص TechLab *Entamoeba histolytica* II ELISA للكشف عن الاميبا الحالة للنسيج ومقارنتها مع الفحص المجهرى .

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* M.Sc. Medical Technology / Hospital Kamal al-Samarrai

** Assistant Prof. / Health and Medical Technical College /Baghdad

*** Assistant Prof. / College of Medicine / University of Nahrain

تم جمع 80 عينة خروج من اشخاص مصابين باحدى الطفيليات المعوية وتتضمن الدراسة 47 ذكر و 33 أنثى تتراوح اعمارهم (1-60) سنة. تم فحص العينات بواسطة الفحص المجهرى المباشر ، بعد ذلك تم الكشف عن المستضد (adhesin) الخاص للاميبيا الحالة للنسج في عينات الخروج باستعمال فحص *TechLab Entamoeba histolytica* II ELISA .

اظهرت النتائج انه من مجموع 80 عينة خروج فحصت مجهريا كانت 65 عينة مصابة باحد انواع الاميبيا (*E. histolytica, E. dispar, E. hartmanni, E. coli*) وعند فحص 65 (81.3 %) بواسطة فحص II *TechLab E. histolytica* ELISA وجد انه 13 (16.3%) مصابة بطفيلي الاميبيا الحالة للنسج بينما 2 (2.5%) كانت مصابة بطفيلي الاميبيا الحالة للنسج باستعمال فحص *TechLab E. histolytica* II ELISA من مجموع 15 (18.8%) عينة كانت سالبة بالفحص المجهرى ($Kappa=0.029$). و بينت الدراسة ايضا انه كانت نسبة الإصابة بأحد انواع الاميبيا اكثر في الذكور (36) وبنسبة مئوية (45.0%) واقل في الأناث (29) وبنسبة مئوية (36.3%) كذلك بالنسبة للاميبيا الحالة للنسج كانت الإصابة في الذكور اكثر (9) وبنسبة مئوية (11.3%) واقل في الأناث (6) وبنسبة مئوية (7.5%). بينت النتائج ان نسبة الإصابة بأحد انواع الاميبيا وكذلك الاميبيا الحالة للنسج كانت ضمن الفئة العمرية (30-39) سنة وبنسبة مئوية (22.5%) ، (7.5%) على التوالي.

الاستنتاجات:

1. قلة خصوصية الفحص المجهرى المباشر في تشخيص داء الاميبيات.
2. الكشف عن المستضد في عينة الخروج باستعمال *TechLab E. histolytica* II ELISA اكثر دقة في تشخيص الاميبيا الحالة للنسج وبالتالي تشخيص داء الاميبيات.

Introduction:

E. histolytica which is the causative agent of amoebic dysentery (amoebiasis) is widely distributed around the world. About 50 million people has become infected a year and eventually 100,000 people lose their lives [1]. Amoebiasis is placed as the second leading cause of death from parasitic diseases world wide. The prevalence of amoebiasis varies with the population affected, differing between countries and between areas with different socioeconomic conditions[2]. Human is considered as a main source of infection. Infection occur by ingestion of cyst with fecal contaminated material [2]. *Entamoeba histolytica* can cause invasive intestinal and extra intestinal disease , on the other hand the indistinguishable species *E. dispar* and other species can not [3]. Thus the correct identification of this parasite is very important since *E. histolytica* is the only species within the genus *Entamoeba* that require treatment. The diagnosis of amoebic colitis rests on the demonstration of *E. histolytica* in the stool or colonic mucosa of the patients. The diagnosis by microscopic identification of the parasite in stool is insensitive and unable to distinguish the invasive parasite *E. histolytica* from the commensal parasite *E. dispar* [4].

After many studies, it is obvious that culture is more sensitive than microscopy, stool culture followed by isoenzyme analysis enable the differentiation of *E. histolytica* from *E. dispar*. However, isoenzyme analysis requires one to several weeks to obtain the result and also special laboratory facilities are required; making it impractical for use in the routine diagnosis of intestinal amebiasis [5]. A number of assays have been developed during recent

years, such as serological methods and DNA detection systems, which are able to distinguish *E. histolytica* from *E. dispar*. Efforts to improve the diagnostic testing have been developed in recent years. Antigen detection assays have proved to be very useful in the diagnosis of some parasitic infections, including *E. histolytica* and *E. dispar* [6]. A number of researchers have reported the detection of amoebic antigen in stool samples to be sensitive and specific [7]. Antigen-based ELISA has many significant advantages for the diagnosis of amoebiasis. Some of the assays are able to differentiate *E. histolytica* from *E. dispar* as TechLab *E. histolytica* II; and have excellent sensitivity and specificity. They can be performed by none expert laboratory technicians and outperform microscopy in their potential as large-scale screening tools in epidemiology studies [8]. Recently, molecule-based PCR assays have been reported to demonstrate excellent sensitivity and specificity compared with microscopy [9]. In several evaluation studies, similar sensitivities and specificities were reported for PCR and ELISA [10]. As PCR techniques are not widely available and remain impractical tools in many developing countries, stool antigen assays are considered valid alternative diagnostic methods for the diagnosis of *E. histolytica* infections.

Materials and Methods:

Stool samples with different consistency form were collected from 80 patients with intestinal protozoa, including 47 male and 33 female , age between (1-60) years. All patients were attending the out patient clinic at Al-Kadhimiya Teaching Hospital / Baghdad for the period from November 2011 to end April 2012.

The stool samples were collected in sterile clean and dry plastic cups with tight lids specially made for this purpose , then each sample were divided into two parts; the first was used for general stool examination and the second part was used for antigen analysis which was stored at -20 °C. Each cup was given a unique name representing the patient. Every patient was reported through a specifically prepared questionnaire which include name , sample number , age, gender , address and others.

Methods:

Laboratory examination include the following :

- 1- General stool examination which include macroscopic examination and microscopic examination.
- 2- Antigen analysis which include detection of *E. histolytica* antigen in stool samples by used TechLab *E. histolytica* II ELISA test.

Note:- The statistical analysis that used include description statistics including statistical tables (Mean, SD, SE, 95% interval confidence) and the strength of agreement between microscope and TechLab *E. histolytica* II ELISA were done by Kappa statistic.

Examination by Microscope:

In macroscopic examination the stool samples were examined by naked eyes for investigating color, consistency , blood , mucus and odor while in microscopic examination which include direct physiological normal saline smear and direct Lugol's- Iodine solution smear.

Principle of ELISA:

Detection of *E. histolytica* antigen was performed by ELISA according to manufacturers instruction. The *E. histolytica* II test (Wampole™ *E. HISTOLYTICA* II, TECHLAB) uses antibodies to the adhesin. The microassay wells contain immobilized polyclonal antibody that bind adhesin of *E. histolytica* /*E. dispar*. The conjugate is a monoclonal antibody-peroxidase conjugate specific for *E. histolytica* adhesin. In the assay, a fecal specimen is emulsified in diluent and the diluted specimen is transferred to a microassay well. If adhesin is present in the specimen, it bind to the conjugate and immobilized polyclonal antibody during the incubation phase. Any unbound material is removed during the washing steps. Following the addition of substrate, a color develops due to the enzyme –antibody-antigen complexes that form in the presence of adhesin [11].

Results : This study was carried on 80 stool specimens with intestinal protozoa (acute and chronic).

Table (1):-Using direct microscopy, intestinal amoebae were detected in 65 out of 80 stool samples (81.3%). Using TechLab *E. histolytica* II ELISA test, *E. histolytica* were detected in 13 out of 65 positive stool samples (by direct microscopy) representing 16.3%, and 2 out of 15 negative stool samples (by direct microscopy) representing 2.5%.

Table 1 : *E. histolytica* samples detected by Microscopy and TechLab ELISA

Microscopic examination	Total No.(%)	TechLab <i>E. histolytica</i> II ELISA	
		Positive No.(%)	Negative No.(%)
Positive	65(81.3)	13(16.3)	52(65.0)
Negative	15(18.8)	2(2.5)	13(16.3)
Total	80(100.0)	15(18.8)	65(81.3)

Kappa= 0.029

The strength of agreement is considered to be 'poor'.

Table (2):- Shows the distribution of intestinal amoebae including *E. histolytica* as detected by direct microscopy according to gender. The majority of intestinal amoebae occur in males 36 (45.0%) compared to females 29 (36.3%). The frequency of *E. histolytica* as detected by ELISA was also higher in males (11.3%) than females (7.5%).

Table 2: Distribution of Intestinal amoebae including *E. histolytica* infection according to gender

Gender	Intestinal amoebae		<i>Entamoeba histolytica</i>	
	Negative No.(%)	Positive No.(%)	Negative No.(%)	Positive No.(%)
Female	4 (5.0)	29 (36.3)	27 (33.8)	6 (7.5)
Male	11 (13.8)	36 (45.0)	38 (47.5)	9 (11.3)
Total	15 (18.8)	65 (81.3)	65 (81.3)	15 (18.8)
Chi-square P- value	*0.203		**0.913	

* P-value is non significant > 0.05 , ** P-value is non significant > 0.05

Table (3) :- Shows the distribution of intestinal amoebae including *E. histolytica* according to age groups. Intestinal amoebae show the highest percentage at age group (30-39) (22.5%) , as well *E. histolytica* show high percentage at age group (30-39) (7.5%).

Table 3: Distribution of Intestinal amoebae including *E. histolytica* infection according to age groups/ years

Age groups/years	Intestinal amoebae		<i>Entamoeba histolytica</i>	
	Negative No.(%)	Positive No.(%)	Negative No.(%)	Positive No.(%)
(> 10)	3 (3.8)	10 (12.5)	11(13.8)	2 (2.5)
(10-19)	0(0.0)	7 (8.8)	7 (8.8)	0 (0.0)
(20-29)	3 (3.8)	6 (7.5)	7 (8.8)	2 (2.5)
(30-39)	0 (0.0)	18 (22.5)	13 (16.3)	6 (7.5)
(40-49)	4 (5.0)	7 (8.8)	10 (12.5)	1 (1.3)
(50-60)	5 (6.3)	17 (21.3)	17 (21.3)	4 (5.0)
Total	15 (18.8)	65 (81.3)	65 (81.3)	15 (18.8)
Chi-square P- value	*0.115		**0.530	

* P-value is non significant > 0.05 , ** P-value is non significant > 0.05

Discussion:

Entamoeba histolytica, one of the two *Entamoeba* species with similar morphology that infect humans, causes invasive intestinal and extra intestinal diseases, whereas *E. dispar* is found commensally and is non-invasive. Because of their morphologic similarity, *E. histolytica* and *E. dispar* cannot be differentiated microscopically. The antigens of *E. histolytica* and *E. dispar*, however, may be detected by the ELISA method [12].

Table (1) summarize the result which reveals that (81.3%) were positive by microscopy. On the other hand, using TechLab *E. histolytica* II ELISA assay, as a gold stander test, the antigen was detected in only (18.8%) sample. Using Kappa statistic, the strength of agreement between these two methods is considered to be "poor". Such results were confirmed by many other studies using the same kit (Wampole™ *E. HISTOLYTICA* II, TECHLAB) [13, 14]. This is because of the low specificity of direct microscopy in confirming the pre diagnosis of amoebiasis. It was stated that the sensitivity and specificity of microscopy ranged between 5%-60% and 10% to 50% respectively [6].

The diagnosis by microscope is time and labour intensive and require experienced microscopists due to the difficulty in differentiating the parasites from leucocytes and other luminal protozoa (*Entamoeba coli*, *Blastocystis hominis*, *Giardia intestinalis*, *Iodamoeba butschlii*) and the inability to different pathogenic species from non pathogenic forms which limits its reliability. Although different results were recorded by other researchers [3, 15] which could be attributed to the use of non specific ELISA kit using polyclonal antibody for detection antigen in stool samples.

Data obtained in this study is demonstrated in table (2) which showed that high infection rate with intestinal amoebae and *E. histolytica* was recorded among male (45.0%), (11.3%) and female (36.3%), (7.5%) respectively. Similar result was found in a study performed in Basrah, Iraq [16]. These results may be explained as males, in fact, consume faecally contaminated food outside more than females. But different results were recorded by other

researchers in Erbil province, Kurdistan region- Iraq and Saudi Arabia [17,18]. Such different results could be due to several factors like socioeconomic, social behavior, residence, and sample size.

The age group (30-39) year showed an infection rate with intestinal amoebae and *E. histolytica* (22.5%), (7.5%) respectively. (Table 3) which is the highest percentage. This result is in agreement with other study because deterioration of the standard of personal hygiene and sanitary conditions in these groups. Also because of the use of human feces as soil fertilizers which increases the chance of spreading infection, and horticulture practice, most of whom are of this age group was also suspected [19].

While other studies, done in Iraq and other regions, found high incidence rates of *E. histolytica* infection among children with age group(4-6) years and primary school children (10-12) years (52.8% and 50.0%) respectively [17]. Protozoan infections were, generally, higher among preschool-children (1-4) years and high incidence rate with *E. histolytica* /*E. dispar* infection was observed among age group (10-19) years [20,21]. Which could be attributed to contamination of milk, drinking water and personal hygienic measures as defecation practices.

It can be concluded that microscopic examination is non specific in the differentiation between pathogenic form of *E. histolytica* from non pathogenic. Thus it is recommended to apply different immunological techniques to confirm the diagnosis.

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