

Differentiation between *Entamoeba histolytica* and *E. dispar* using Enzyme-linked Immunosorbent Assay and Wet Mount Method

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Abstract

To differentiate between *Entamoeba histolytica* and *E. dispar* among patients attended Pediatric, General Hospital and Primary Health Care Centres in Kirkuk City, using Direct wet mount and ELISA techniques. The current study included the examination of (600) stool specimens of patients of different age groups (1-≤60 years) attended Pediatric Hospital, Kirkuk General Hospital, and Primary Health Care Centers in Kirkuk city for the period from 1/8/2008 to 20/4/2009 to search for the prevalence of *Entamoeba histolytica* and *Entamoeba dispar* using ELISA. It was found that out of 600 stool specimens examined by wet mount microscopy 25.66 % were infected with *E. histolytica* / *E. dispar*. DRG ELISA based antigen detection kit of *E. histolytica* / *E. dispar* in stool specimens revealed that 52.12 % were found to be positive of total specimens examined (140 microscopy positive samples and 48 microscopy negative control samples). TechLab ELISA based antigen detection kit specific only for *E. histolytica* in stool specimens revealed that 6.38 % were positive for *E. histolytica*, while the remaining negative 93.62 % were considered *E. dispar*. DRG ELISA based antibody detection of anti-*E. histolytica* serum IgG revealed seropositivity rate of 36.17 %. The direct stool examination is not capable to differentiate between *E. histolytica* and *E. dispar*. TechLab ELISA based antigen detection of *E. histolytica* is a sensitive and rapid method for detection and differentiation of *E. histolytica* from *E. dispar*. DRG ELISA based antibody detection of anti-*E. histolytica* serum IgG is a helpful mean to detect chronic infection of amebic colitis, asymptomatic cyst carrier patients, and extra-intestinal infection.

التمييز بين اميبا الزحار واميبا دسبار باستخدام تقنية الاليزا والمسحة الرطبة

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الملخص

تضمنت الدراسة الحالية فحص 600 عينة براز للمرضى المراجعين لمستشفى الاطفال ، مستشفى كركوك العام ، ومراكز الرعاية الصحية الاولى في مدينة كركوك للفترة من 2008/8/1 ولغاية 2009/4/20 للتحري عن نسبة الخمج لاميبا الزحار واميبا الدسبار باستعمال طريقة ELISA . اظهرت الدراسة الحالية نسبة خمج 25.66% (154 من 600) بأميبا الزحار/أميبا الدسبار فحصت بطريقة المسحة المباشرة باستخدام المجهر كشف الممنع المرتبط بالانزيم DRG (ELISA) القائم على كشف المستضدات لأميبا الزحار /أميبا الدسبار في عينة البراز عن نسبة خمج ايجابية في 52.12% (98 من 188) من مجموع العينات الكلية المفحوصة (140 عينة ايجابية مجهريا و 48 عينة سيطرة سلبية مجهريا). أما الفحص الممنع المرتبط بالانزيم (TechLab ELISA) القائم على كشف المستضدات المتخصصة لأميبا

الزحار فقد كشف عن نسبة خمج ايجابية في 6.38% ، اما بقية العينات التي اعطت تفاعلا سلبيا فكانت نسبتها 93.92% اعتبرت أميبا الدسبار . واطهر الفحص الممنع المرتبط بالانزيم (DRG ELISA) القائم على كشف الاضداد من نوع IgG لأميبا الزحار في العينات المصلية نسبة اصابة ايجابية في 36.17% (34 من 94). يستنتج مما ذكر اعلاه ان الفحص الممنع المرتبط بالانزيم (TechLab ELISA) القائم على كشف المستضدات المتخصصة لطفيلي أميبا الزحار ، يعد فحصا دقيقا وطريقة سريعة للكشف والتفريق بين أميبا الزحار و أميبا الدسبار . اما الفحص الممنع المرتبط بالانزيم (DRG ELISA) القائم على كشف الاضداد من نوع IgG لطفيلي أميبا الزحار ، يعد وسيلة تساعد في الكشف عن الاصابات المزمنة بالزحار الاميبي المعوي، وللكشف عن الاشخاص المصابين الحاملين للطور المتكيس الذين لا تظهر عليهم اعراض المرض، وللكشف عن الاصابات الخارج معوية.

Introduction

Entamoeba histolytica has been differentiated from *E. dispar* on the basis of genetic difference, both protozoa are morphologically identical but have genetic and functional differences, *E. histolytica* is invasive and causes disease such as colitis and liver abscess while *E. dispar* causes asymptomatic colonization which does not need to be medically treated (1). The diagnosis of amoebic colitis rests on the demonstration of *E. histolytica* in the stool or colonic mucosa of patients (2). The diagnosis of amoebiasis 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* (3). Because microscopy is unable to distinguish between these two organisms, it should no longer be relied upon to diagnose amoebiasis. Sensitive and specific molecular techniques that are able to distinguish *E. histolytica* from *E. dispar* have been developed recently, these methods include the detection of an *E. histolytica* antigen using an enzyme-linked immunosorbent assay (ELISA), the use of the polymerase chain reaction (PCR) to amplify amoebic DNA, and the culture of stool samples followed by isoenzyme analysis (4). Several molecular diagnostic tests, including serological techniques that have been used so far for immunodiagnosis of amoebiasis, these includes indirect haemagglutination (IHA), counterimmuno-electrophoresis (CIE), amoebic gel diffusion test, complement fixation (CF), indirect fluorescence assay

(IFA), latex agglutination, enzyme linked-immunosorbent assay (ELISA) (5). The study was planned to differentiate between *E. histolytica* and *E. dispar* using Enzyme-Linked Immunosorbent Assay (ELISA) in addition to the Conventional Method.

Materials and Methods

Study population

The study conducted from 1/8/2008 to 20/4/2009. Stool samples were collected from 600 patients of different age groups ($\leq 1-60$ years) who complain of diarrhea and/or abdominal discomfort attended the Pediatric and General Hospital and Primary Health Care Centers in Kirkuk city. Collection of stool samples. Stool samples were collected using a sterile wide mouth screw cap containers, fresh samples were examined by direct wet mount technique. Small amount (0.5 ml - 3 ml) of stool specimens were collected in sterile screw cap containers and kept at -20°C using (VestFrost-Denmark) refrigerator until being examined by ELISA, Microscopy positive samples were further examined by DRG ELISA based antigen detection of *E. histolytica* / *E. dispar* in stool specimens, positive DRG ELISA samples were further tested with TechLab *E. histolytica* II monoclonal ELISA based antigen detection in stool specimen, and 94 serum samples were examined by *E. histolytica* serum IgG ELISA. Collection of blood samples, Blood samples were collected from patients whom stool examinations were positive for the presence of *E. histolytica* / *E. dispar* trophozoites and/or

cysts and from control healthy persons using sterile syringes. The blood specimens then voided into a sterile screw cap serum tubes and allowed to coagulate then centrifuged for 3-5 minutes at 3000 rpm then serum were collected into sterile screw cap serum tubes and kept at -20°C until being examined by ELISA for *E. histolytica* serum IgG. Examination of stool specimens, Stool samples were examined by wet mount preparation using normal saline 0.9 %, buffered methylene blue, and lugol's iodine 1 % (6). The DRG ELISA stool antigen assay was performed on 140 microscopy positive stool specimens and 48 control stool specimens (microscopy negative for *E. histolytica*/ *E. dispar*) according to manufacturing company (DRG Instruments GmbH, Germany). The monoclonal ELISA for detecting *E. histolytica* adhesin in fecal specimen was performed on 94 stool specimens that were positive by DRG ELISA *E. histolytica* / *Entameoba dispar* stool

antigen, according to manufacturing company (TECHLAB Inc., Blacksburg, Virginia, USA).

Results

Out of 600 stool specimens examined by microscopy (wet mount), *E. histolytica* / *E. dispar* were found in 154 (25.66 %) patients. Table (1) shows that out of 188 stool samples including 140 samples positive by microscopic examination and 48 negative samples, it was found that 97 (69.29 %) samples out of 140 were positive by DRG ELISA and 43 (30.71 %) were negative, while only 1 (2.08 %) sample was positive for *E. histolytica* / *E. dispar* in control samples. The overall rate positive DRG ELISA stool antigen among 188 stool samples was 98 (52.12 %). The DRG stool ELISA shows sensitivity of (69.28 %), specificity of (97.91 %) and predictive value of (97.8 %).

Table (1):- Detection of *E. histolytica* / *E. dispar* antigen in stool samples by DRG ELISA

Examination Method	No. Samples	+ve	%	-ve	%
Microscopy positive samples tested by DRG ELISA	140	97	69.29 %	43	30.71%
Control negative	48	1	2.09 %	47	97.91%
Total	188	98	52.12 %	90	47.88%
Sensitivity	69.28 %				
Specificity	97.91 %				
Predictive value	97.80%				

Table (2) indicates that out of 94 stool samples that were positive for *E. histolytica* / *E. dispar* by DRG ELISA, only 6 (6.38 %) were positive for *E.*

histolytica while 88 (93.62 %) stool samples were negative and considered *E. dispar*.

Table (2):- Detection of *E. histolytica* antigen in stool samples by TechLab ELISA technique.

Examination method	No. samples	+ve	%	-ve	%
TechLab ELISA <i>E.histolytica II</i>	94	6	6.38 %	88	93.62 %

Table (3) shows that serum IgG antibody against *E. histolytica* was detected in all 6 (100.0 %) serum samples of patients positive *E. histolytica* TechLab stool ELISA, and 24 (37.50 %) sera of patients positive *E. histolytica* / *E. dispar* DRG stool

ELISA, while, only 4 (16.66 %) serum samples were positive out of 24 control samples, and 20 (83.34 %) were negative. The overall seropositive rate of serum IgG for *E. histolytica* among 94 samples was 34 (36.17 %).

Table (3):- Serum IgG for *E. histolytica* among positive DRG ELISA, TechLab ELISA and control serum samples.

Examination Method	No. samples	+ve	%	-ve	%
Serum samples from DRG positive stool	64	24	37.50 %	40	62.50 %
Serum sample from TechLab positive Stool	6	6	100.0 %	0	0 %
Control samples microscopy negative	24	4	16.67%	20	83.33 %
Total	94	34	36.17%	60	63.83%

Table (4), indicates that the rate of positive stool samples for *E. histolytica* and *E. dispar* by microscopic examination was 25.66 %; in DRG ELISA stool antigen the positive rate was 52.12 % and negative

47.88%; in TechLab ELISA stool antigen positive rate for *E. histolytica* was 6.38 %, while negative was 93.62% . In DRG ELISA serum IgG the positive rate was 36.17 %, while negative 63.83% respectively.

Table (4):- The positivity rates using different diagnostic methods for *E. histolytica*

Examination method	No. Samples	+ve	
Microscopy wet mount	600	154	25.66 %
DRG <i>E. histolytica</i> / <i>E. dispar</i> stool antigen	188	98	52.12 %
TechLab <i>E. histolytica</i> II stool antigen	94	6	6.38 %
DRG <i>E. histolytica</i> Serum IgG	94	34	36.17 %

Discussion

Percentage of *E. histolytica* / *E. dispar* in stool specimens examined by microscopy

The rate of infection with *E. histolytica* / *E. dispar* were 25.66 % from total of 600 stool samples examined by wet mount, this result correlates with the study conducted by Shebib *et al.* (7) who recorded infection rate of 25 % of *E. histolytica* in children in Baghdad city. This result is also approach to that recorded by AL-Yassaree (8) who recorded infection rate of 29.5 % in Babylon city. This is also near to AL-Samarray (9) in Samara district, Salahaddin governorate, who recorded infection rate of 20.74%.

Antigen detection ELISA in stool specimens

The DRG ELISA *E. histolytica* / *E. dispar* kit recorded infection rate of 52.12 % (98 of 188). The result agreed with AL-Harathi and Jamjoom (10) in Saudi Arabia who recorded 59.6 % (112 of 156) infection rate for *E. histolytica* / *E. dispar* using Triage Parasite Panel. It was contrary with Delialioglu *et al.* (11) in Turkey who recorded 29.5 % (26 of 88) infection rate for *E. histolytica* / *E. dispar* using Ridascreen *Entamoeba* ELISA kit. The

lower DRG ELISA detection compared to microscopy may be due to misdiagnosis results by microscopic examination resulting from confusing of polymorphonuclear leukocytes or macrophages with trophozoites and cysts of *E. histolytica*, or confusion with cysts of different species of *Entamoeba* such as *E. hartmanni*, *Endolimax*. The lower detection rate by ELISA may be due to low density of trophozoites in stool specimen (12). The variation of ELISA results may be attributed to the fact that different ELISA kits were used from different companies, each differs in sensitivity, this difference may be attributed to the quantity of the parasites presented in stool or the presence of *E. moshkovskii* which is indistinguishable in its cyst and trophozoite from *E. histolytica* / *E. dispar*, which cannot be captured by the DRG ELISA kit. Regarding TechLab *E. histolytica* II ELISA kit, the recorded infection rate 6.38 % for *E. histolytica*, and 93.62 % for *E. dispar*. This result is in agreement with AL-Harathi and Jamjoom (10) in Saudi Arabia who recorded infection rate 4.3 % of *E. histolytica*, and 95.7 % for *E. dispar* using TechLab *E. histolytica* II ELISA kit, Kurt *et al.* (13) in Turkey

reported higher infection rate 88.1 % for *E. dispar* compared to *E. histolytica* 8.5 % using TechLab *E. histolytica* II ELISA kit, while Mohammadi *et al.* (14) in Iran recorded 98.9 % for *E. dispar*, these 88 microscopy positive samples were submitted to TechLab ELISA and all were negative for *E. histolytica*. The result also closes to Haque *et al.* (15) in Bangladesh who recorded infection rate 4.29 % for *E. histolytica* using TechLab *E. histolytica* II ELISA kit, and to Nesbitt *et al.* (3) in Tanzania who reported infection rate 0.8 % for *E. histolytica* and 7.4 % for *E. dispar* using TechLab *E. histolytica* II ELISA kit. Braga *et al.* (16 and 17) in Northeastern Brazil recorded higher rate 10.2 % (41 of 401), and 58.9 % (110 of 187) for *E. histolytica* compared to 9.2 % (37 of 401), and 41.1 % (77 of 187) for *E. dispar* respectively, using TechLab *E. histolytica* II ELISA kit. The high rate of *E. dispar* recorded in the current study may be due to the fact that *E. dispar* is the predominant *Entamoeba*. Of the vast number infected, the greater majority are infected with *E. dispar*, which explains, in part, the low percentage of disease symptoms in infected persons. It estimated that only 10 % of reported cases are due to *E. histolytica*. A study in Kilimanjaro / Tanzania, indicated that *E. dispar* infection was 14.4 time more prevalent than *E. histolytica* infection (18). In the current study the fewer patients included with bloody diarrhea, this may reflect the lower rate recorded for *E. histolytica*, in addition, the virulent strain of *E. histolytica* may differs according to geographical and environmental factors. The remaining majority reported with *E. dispar* which is a non pathogenic *Entamoeba*, complain of abdominal discomfort or diarrhea, this may be attributed to the association of *E. dispar* with other

diarrheal causing agent (*Rota virus*, *Shigella* species, *Campylobacter* species enterohemorrhagic or enteroinvasive *Escherichia coli*, and *Salmonella* species) so the commonly reported complaints of diarrhea require alternative explanation. ELISA for *E. histolytica* fecal adhesins permits rapid detection and can be used for specimens submitted for routine clinical testing from adults or children. In addition ELISA test is highly sensitive, as little as 0.2 - 0.4 ng of parasitic antigen can be detected from stool samples (18). *E. histolytica* Serum IgG Antibody detection ELISA The serum IgG antibody against *E. histolytica* was detected in 36.17 % using DRG *E. histolytica* serum IgG ELISA . This result is close to Haque *et al.* (19) in Bangladesh who recorded 32.7 % (in a total of 232 children) seropositivity for *E. histolytica* serum IgG using TechLab ELISA, but was lower than Abd-Alla *et al.* (20) in Egypt who recorded 56.3 % seropositivity in patients with acute colitis using ELISA technique. The high anti-*E. histolytica* seropositivity rate may be due the persistence of IgG antibodies for years after infection, or due to the incomplete drug treatment, or the antibody was due to an extraintestinal infection (amoebic liver abscess or other sites), or individuals may be constantly reinfected throughout their lives, or continual exposure to the parasite or there may be no immunity or incomplete immunity to colonization with *Entamoeb histolytica* (11 and 19). *E. dispar* infection is not associated with the production of antibodies and this may reflect the noninvasive character of *E. dispar*, in contrast, one hundred percent of the patient infected with *E. histolytica* had serum antibodies (19). A possible explanation for these conflicting studies is that there may be restricted invasiveness of some strains

of *E. histolytica* and that these strains may consequently fail to elicit a circulating-antibody response. This may be reflected in the low incidence of invasive amoebiasis found in these communities. Also, since this study was a one-time sampling, the period of colonization with *E. histolytica* / *E. dispar* was not known (16). It is concluded that light microscopy is not capable of differentiating *E. histolytica* from *E. dispa*. The antigen detection ELISA tests for the *E. histolytica*-*E. dispar* complex is reliable and sensitive for the differentiation of *E. histolytica* from *E. dispar* in stool specimens. The rate of *E. histolytica* infection was lower than *E. dispa* using DRG ELISA. Serological methods that detect anti-*E. histolytica* IgG antibodies can be used to distinguish between infection with *E. histolytica* and *E. dispar*. It is recommended to differentiate between *E. histolytica* and *E. dispar* with *E. histolytica*-specific antigen in stool samples and detection of anti *E. histolytica* IgM and IgG antibodies by serological methods.

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