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Diagnosis of Amebic Liver Abscess and Intestinal Infection with the TechLab *Entamoeba histolytica* II Antigen Detection and Antibody Tests

RASHIDUL HAQUE,[1] NASIR UDDIN MOLLAH,[2] IBNE KARIM M. ALI,[2] KHORSHED ALAM,[2] ALEIDA EUBANKS,[3] DAVID LYERLY,[3] AND WILLIAM A. PETRI, JR.[4]*

Centre for Health and Population Research, ICDDR,B,[1] and Department of Microbiology and Hepatology, Bangabandhu Sheikh Mujib Medical University,[2] Dhaka, Bangladesh, and TechLab, Inc., Blacksburg,[3] and Departments of Medicine, Microbiology and Pathology, University of Virginia, Charlottesville,[4] Virginia

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A noninvasive diagnostic test for amebic liver abscess is needed, because amebic and bacterial abscesses appear identical on ultrasound or computer tomography and because it is rarely possible to identify *Entamoeba histolytica* in stool specimens from patients with amebic liver abscess. Here we report a method of detection in serum of circulating *E. histolytica* Gal/GalNAc lectin to diagnose amebic liver abscess, which was used in patients from Dhaka, Bangladesh. The TechLab *E. histolytica* II test (which differentiates the true pathogen *E. histolytica* from *Entamoeba dispar*) detected Gal/GalNAc lectin in the sera of 22 of 23 (96%) amebic liver abscess patients tested prior to treatment with the antiamebic drug metronidazole and 0 of 70 (0%) controls. After 1 week of treatment with metronidazole, 9 of 11 (82%) patients became serum lectin antigen negative. The sensitivity of the *E. histolytica* II antigen detection test for intestinal infection was also evaluated. Antigen detection identified *E. histolytica* infection in 50 samples from 1,164 asymptomatic preschool children aged 2 to 5 years, including 16 of 16 (100%) culture-positive specimens. PCR analysis of stool specimens was used to confirm that most antigen-positive but culture-negative specimens were true-positive: PCR identified parasite DNA in 27 of 34 (79%) of the antigen-positive, culture-negative stool specimens. Antigen detection was a more sensitive test for infection than antilectin antibodies, which were detected in only 76 of 98 (78%) amebic liver abscess patients and in 26 of 50 (52%) patients with intestinal infection. We conclude that the TechLab *E. histolytica* II kit is a sensitive means to diagnose hepatic and intestinal amebiasis prior to the institution of metronidazole treatment.

Amebiasis is a common worldwide disease caused by the protozoan parasite *Entamoeba histolytica*; 100,000 people are estimated to die each year from amebic colitis and amebic liver abscess (30). In recent years, sensitive and specific test methods for the diagnosis of intestinal amebiasis have been developed. These stool sample tests differentiate the true pathogen *E. histolytica* from the identical-appearing *Entamoeba dispar* and include parasite antigen and DNA detection by enzyme immunoassay (EIA) and PCR, respectively (4, 10). However, the use of these techniques for diagnosis of amebic liver abscess is mostly unexplored.

The diagnosis of amebic liver abscess is sometimes difficult since its clinical manifestations are highly variable. In areas of endemicity, amebic liver abscess should always be suspected in a patient with fever, weight loss, and right upper quadrant abdominal pain and tenderness. Imaging techniques such as ultrasound, computed tomography, and magnetic resonance have excellent sensitivity for the detection of liver abscess arising from any cause but cannot distinguish amebic abscesses from pyogenic (bacterial) abscesses or necrotic tumors. Most patients with an amebic liver abscess do not have coexistent amebic colitis. Therefore, stool microscopy or antigen detection in stool samples is not helpful for diagnosis: less than 10% of patients have identifiable amebae in stool (17).

Serological tests demonstrate the presence of antiamebic antibodies in serum and are positive for most patients with amebic liver abscess. A drawback of serologic tests that detect antibodies against total amebic antigens is that individuals in areas of endemicity can remain positive for years after infection (7, 14, 15, 18, 31). In contrast, the antibody response to the Gal/GalNAc adherence lectin appears to be shorter lived, and limited experience in South Africa suggests that it is a more specific serologic test for acute amebiasis (1, 2, 6, 20–22, 24, 32).

Several groups have reported the detection of amebic antigen in the serum of liver abscess patients (1, 16). For example, Abd-Alla and colleagues detected the Gal/GalNAc lectin in the sera of 75% of South African patients with amebic liver abscess (1). A commercially available antigen detection test, the TechLab, Inc. (Blacksburg, Va.), *E. histolytica* test, detects the Gal/GalNAc lectin in stool samples and has proven to be a sensitive and specific means of diagnosis of colitis (11–13). A second-generation kit that uses an improved capture antibody has recently been developed by TechLab. This test had never before been evaluated for detection of lectin antigen in the serum of amebic liver abscess patients. In this study, we evaluated this improved antigen detection kit and an antilectin antibody detection test, both supplied by TechLab, for the diagnosis of amebic liver abscess and intestinal infection in Dhaka, Bangladesh.

MATERIALS AND METHODS

Subjects. The subjects in the present study included 98 patients with amebic liver abscess who were admitted to different private and public hospitals of the city of Dhaka, as well as 70 controls and 1,164 preschool children aged 2 to 5 years from Mirpur, an urban slum in Dhaka. Of the 98 amebic liver abscess patients, 75 had received treatment prior to measurement of serum antigen.

* Corresponding author. Mailing address: P.O. Box 801340, University of Virginia Health System, Charlottesville, VA 22908-1340. Phone: (804) 924-5621. Fax: (804) 924-0075. E-mail: wap3g@virginia.edu.

Most of the patients and controls were from neighboring districts of Dhaka. Informed consent was obtained from the patients and parents of the children. The Ethical Review Committee of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), and the Human Investigation Committee of the University of Virginia reviewed and approved the study design. The control group included 70 individuals whose distribution by age, sex, and profession was similar to that of the patients with amebic liver abscess but who had no history of recent dysentery or diarrhea and whose stool samples were negative for *E. histolytica* infection by culture and stool antigen detection test. Forty-six of the controls were healthy asymptomatic volunteers, and the other 24 were patients with either viral hepatitis or pyogenic liver abscess (defined by a positive bacterial culture of an aspirate from a space-occupying hepatic lesion).

The diagnosis of amebic liver abscess was based on four or more of the following criteria: (i) a space-occupying lesion in the liver diagnosed by ultrasonography and suggestive of abscess, (ii) clinical symptoms (fever, pain in the right hypochondrium (often referred to the epigastrium), lower chest, back, or tip of the right shoulder), (iii) enlarged and/or tender liver, usually without jaundice, (iv) raised right dome of the diaphragm on chest radiograph, and (v) improvement after treatment with antiamebic drugs (e.g., metronidazole). Where available, information from the liver abscess aspirate was also used for the diagnosis. A total of 36 liver abscess aspirates were performed. Of these 36, 27 were diagnosed as amebic liver abscess, including 5 amebic liver abscesses with concomitant bacterial infection. Of the 27 aspirates diagnosed as amebic, 25 were positive for either antilectin antibody or antigen, including all 5 that were also infected with bacteria. The other nine aspirates were diagnosed as pyogenic based on positive aspirate bacterial cultures and negative amebic antigen and antibody in the liver abscess aspirates. The nine pyogenic patients did not respond to metronidazole and had an approximately equal ratio of males to females.

Sample collection. Stool samples were collected from all the subjects of this study. Venous blood (5 ml) was collected from each amebic liver abscess patient, each control subject, and each of the 1,164 preschool children; sera were separated and stored at −20°C until used. A detailed history of prior treatment with antiamebic drugs and antibiotics was taken at the time of collection of blood from amebic liver abscess patients. Liver aspirate pus was collected from 27 amebic liver abscess patients. Liver abscess pus was aspirated only for clinical purposes as judged by the clinicians caring for the patients and not for the purpose of this study.

Microscopy and culture. Fresh stool samples were examined for the presence of blood that was visible to the naked eye, and a smear of feces in 0.9% saline and Lugol's iodine was examined microscopically for blood and for the presence of *E. histolytica-E. dispar* complex cysts and trophozoites. Stool samples were cultured for *Entamoeba* species in Robinson's medium within 6 h of collection. After 48 h, a drop of culture sediment was examined microscopically for the presence of *E. histolytica-E. dispar* complex trophozoites (25). Liver aspirate pus was also microscopically examined and cultured in Robinson's medium.

PCR test. The PCR for detection of *E. histolytica* infection in stool samples was carried out according to a protocol previously described (10). The nested PCR test was based on the amplification of the small-subunit rRNA gene of *E. histolytica*.

Antigen detection. The TechLab *E. histolytica* II test (designed to detect specifically *E. histolytica* in stool specimens) was performed on the stool specimens according to the manufacturer's instructions. For detection of antigen in the serum samples, 100 μl of undiluted serum was added to the coated microtiter well of the kit. Liver abscess pus specimens were vortexed and centrifuged at 10,000 × *g* for 10 min, and 100 μl of the resulting undiluted supernatant was added to the microtiter well used for antigen detection. A test was considered positive when the optical density reading of a sample was >0.15 at 450 nm (according to the manufacturer's instructions).

ELISA for detection of anti-lectin antibodies. The antilectin immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) procedure was modified from the procedure of Ravdin and colleagues (24). Ninety-six-well microtiter plates were coated with purified lectin (TechLab). Test sera were added at a 1:1,000 dilution in phosphate-buffered 0.9% saline (PBS)–0.1% Tween 20–1% bovine serum albumin (final volume, 100 μl) for 2 h at room temperature. Wells were washed four times with PBS-Tween 20, and the plates were incubated with 100 μl of a 1:5,000 dilution of horseradish peroxidase-conjugated goat anti-human IgG for 1 h at room temperature. The wells were again washed four times in PBS-Tween, followed by the addition of the substrate (10 mg of *O*-phenylenediamine dihydrochloride per ml in 0.1 M citrate phosphate buffer [pH 5.0] containing 0.13% H_2O_2). After development, 1 M sulfuric acid was used as the stop solution. The optical densities of the microtiter wells were measured at 450 nm with an ELISA plate reader (Titertek Multiskan; Flow Laboratories, McLean, Va.). To obtain a high level of specificity, the results were corrected for nonspecific background by subtracting the optical densities from wells in which sera were not added but otherwise exposed to the identical procedure described above. The supernatant of liver abscess pus specimens tested for antilectin antibodies was treated similarly to that of serum samples. A sample was considered positive if the optical density reading was ≥0.5, as was determined in an earlier study (11).

TABLE 1. Detection of lectin antigen and antilectin antibody in serum specimens from amebic liver abscess patients and controls

Detection target	No. (%) of specimens from patient group (*n*) positive for detection target		
	Amebic liver abscess without prior treatment with metronidazole (*n* = 23)	Amebic liver abscess with prior treatment with metronidazole (*n* = 75)	Controls (*n* = 70)
Lectin antigen	22 (95.7)	10 (13.3)	0 (0)
Antilectin antibody	13 (56.5)	63 (84.0)	2 (2.9)

RESULTS

The TechLab *E. histolytica* II test detected Gal/GalNAc lectin in the serum of 22 (96%) of 23 amebic liver abscess patients who were tested prior to treatment with the antiamebic drug metronidazole (Table 1). In contrast, prior metronidazole treatment significantly decreased the ability to detect Gal/GalNAc lectin in the sera from amebic liver abscess patients, with only 10 (15%) of 75 patient specimens positive ($P < 0.001$) (Table 1).

Metronidazole treatment had been initiated from a few days to several weeks before collection of the blood samples in these patients. None of the control subjects were positive for lectin antigen in the serum, but 2 (2.9%) of 70 were positive for antilectin antibody (Table 1). Antilectin antibodies were detected in 76 (78%) of 98 amebic liver abscess patients and were detected in a higher percentage of patients (63 of 75 [84%]) who had received prior treatment with metronidazole ($P < 0.05$) (Table 1).

Out of the 22 patients with serum samples positive for lectin, 11 were available for collection of follow-up blood samples. From these 11 amebic liver abscess patients, blood samples were collected every week for 4 weeks after the initiation of metronidazole treatment. Serum samples were tested for lectin antigen by the *E. histolytica* II test. It was found that after 1 week of treatment with metronidazole, 9 (82%) out of 11 amebic liver abscess patients' samples became serum lectin antigen negative. One patient's blood sample became lectin antigen negative after 2 weeks of treatment with metronidazole. Only one patient continued to be lectin antigen positive until 4 weeks after treatment, and this patient failed to develop a serum antilectin antibody response. This suggests either that this patient was immunosuppressed or that the *E. histolytica* strain was resistant to metronidazole.

Liver abscess pus was collected from 27 amebic liver abscess patients. Microscopy revealed that 3 of 27 (11%) liver abscess specimens were positive for *E. histolytica*, but only 1 yielded growth in culture. Out of 27 liver abscess pus specimens, 11 (41%) were positive for lectin antigen, and 14 (52%) were positive for antilectin antibody (Table 2). It was observed that all three of the liver abscess samples collected prior to treatment with metronidazole were positive with the *E. histolytica* II test. Liver abscess pus specimens that were positive for lectin antigen were not positive for antilectin antibody, with only two liver abscess pus specimens negative for both lectin antigen and antilectin antibody.

Stool samples were obtained from 47 amebic liver abscess patients and tested by microscopy, culture, and the *E. histolytica* II test. Only one stool specimen from an amebic liver abscess patient was positive by both microscopy and culture. Three (42.9%) of seven stool specimens that were obtained from patients without prior treatment with metronidazole were

TABLE 2. Detection of lectin antigen and antilectin antibody in liver abscess pus of amebic liver abscess patients

Detection target	No. (%) of specimens from patient group (*n*) positive for detection target		
	Without prior treatment with metronidazole (*n* = 3)	Prior treatment with metronidazole (*n* = 24)	Total (*n* = 27)
Lectin antigen	3 (100)	8 (33.3)	11 (40.7)
Antilectin antibody	0 (0)	14 (58.3)	14 (51.9)

positive, while three (7.7%) of 40 stool specimens that were obtained from patients during or after treatment with metronidazole were positive for lectin antigen.

Out of 27 amebic liver abscess aspirates examined for bacteria, 22 (81%) aspirates showed no growth, while 5 (19%) aspirates were positive for bacteria, including 3 for *Pseudomonas*, 1 for *Proteus*, and 1 for *Escherichia coli*. Gram stain showed gram-negative rods in these five liver positive abscess aspirates. Only one of the five patients with a mixed bacterial-amebic abscess had received prior percutaneous drainage. Superinfection of amebic liver abscess with bacteria has previously been reported in the literature (9, 26).

Culture and *E. histolytica* II antigen detection were used to test single stool specimens from 1,164 asymptomatic preschool children aged 2 to 5 years (Table 3). Cultures for *E. histolytica* were positive in 16 (1.4%) stool specimens. All of the 16 stool samples that were positive by the culture were also positive by the antigen detection test. In addition, the antigen detection test identified 34 more stool specimens as positive. All of the 50 stool specimens that were positive for *E. histolytica* by the antigen detection test were tested by PCR for *E. histolytica* DNA. PCR identified 43 of the 50 positive specimens as *E. histolytica*, including all 16 stool specimens that were positive by culture. We suspect that most of the 7 of 50 (14%) antigen-positive, culture-, and PCR-negative stool samples are true-positives by the antigen detection test since the PCR test is the least sensitive of the three techniques used (10).

Serum samples from the 1,164 preschool children were also tested for antilectin antibodies (Table 4). There were 171 (14.7%) children who were positive for antilectin antibody among these children. Of these 171 seropositive children, 26 (15.2%) had stool specimens that were also positive for *E. histolytica* by the antigen detection test, while only 24 (2.4%) of 993 seronegative children had stool specimens that were positive for *E. histolytica* infection (Table 4).

TABLE 3. Detection of *E. histolytica* by culture and *E. histolytica* II test in stool specimens of 1,164 preschool children

E. histolytica II test result[a]	No. of patient specimens with reaction in culture for *E. histolytica*[b]		
	Positive	Negative	Total
Positive	16	34[c]	50
Negative	0	1,114	1,114
Total	16	1,148	1,164

[a] *E. histolytica* was detected using *E. histolytica* II fecal antigen detection test (TechLab, Inc.).

[b] Fecal specimens were cultured in Robinson's medium.

[c] *E. histolytica* was detected by PCR in 27 of these 34 culture-negative stool specimens.

TABLE 4. Comparison of the *E. histolytica* II test results in stool specimens and antilectin antibody test results in serum of 1,164 preschool children

E. histolytica II stool test result	No. of specimens with reaction for antilectin antibody in serum		
	Positive	Negative	Total
Positive	26	24	50
Negative	145	969	993
Total	171	993	1,164

DISCUSSION

The major conclusion of this study is that amebic liver abscess can be diagnosed by the detection of circulating antigen. The TechLab *E. histolytica* II kit was able to detect serum antigen (Gal/GalNAc lectin) in almost all patients with amebic liver abscess who had not received treatment with metronidazole. Serum antigen detection was more sensitive than detection of antilectin antibody for the diagnosis of amebic liver abscess prior to treatment with metronidazole. Antigenemia did not persist in amebic liver abscess patients after treatment with metronidazole; 10 of 11 (91%) serum lectin antigen-positive amebic liver abscess patients became negative for serum lectin antigen within 2 weeks after the start of antiamebic treatment. The effect of metronidazole in clearing antigenemia has also been shown in an animal model (28). These results indicate that the TechLab antigen detection test can be used with serum samples to diagnose amebic liver abscess and can also be used as a test of cure. Detection of amebic antigen in serum of amebic liver abscess patients has been reported in the literature and has shown variable sensitivity and specificity (1, 16, 23, 29). This is the first study to use a commercially available antigen detection test, and it has the advantage of the use of a well-defined antigen (the Gal/GalNAc lectin) (19, 27).

Antilectin antibodies were detected in 56.5% of amebic liver abscess patients without prior treatment with metronidazole, while 84% of patients with prior treatment with metronidazole had antilectin antibodies in their blood. This may be due to an antibody response that appeared late in the disease course as antigen disappeared from the blood. The overall sensitivity of the antilectin antibody test was 78%, while that of serum lectin antigen detection was 34.8%. However, antigen detection had a much higher sensitivity (95.7%) when only sera collected prior to treatment were analyzed. Most of the patients with amebic liver abscess in this study had already started treatment with metronidazole when the blood was collected. In developing countries where amebic liver abscess is endemic, antiamebic drugs and antibiotics may be used indiscriminately, making it hard to obtain an accurate treatment history. So for ultimate sensitivity, the use of both lectin antigen detection and antilectin antibody detection in serum may be required for diagnosis of acute amebic liver abscess. In developed countries where metronidazole is not available without a prescription, the use of the *E. histolytica* II kit alone for detection of antigen in serum may prove to be very useful for the diagnosis of amebic liver abscess.

Detection of intestinal infection with the TechLab *E. histolytica* II kit was more sensitive than with the first-generation kit. The first-generation kit was 85% sensitive compared to culture (13), whereas the second-generation kit identified all 16 of the culture-positive samples, as well as 34 additional samples that were culture negative. That most of these additional antigen-positive stool samples were true positives was demonstrated with a PCR test that identified *E. histolytica*

DNA in 27 of 34 samples (79%); earlier it had been demonstrated that this PCR test had 87% sensitivity compared to culture (13).

We also found an association between seropositivity and stool colonization with *E. histolytica*. In our study, 52% of the children that were colonized with *E. histolytica* were seropositive, and 13% of children whose stools were *E. histolytica* negative were seropositive (Table 4). These results contrast with those obtained from Brazil, where no correlation was observed between seropositivity and stool colonization with *E. dispar* and/or *E. histolytica* (3). Our data also differ from those of a study conducted in India that found 12.8% of seropositive individuals to be colonized with *E. histolytica-E. dispar* complex (as defined by microscopic examination of stool), whereas 20.3% of seronegative individuals were colonized (5). Results similar to ours have been reported from South Africa, where it was found that 99% of amebic liver abscess patients and 100% of asymptomatic *E. histolytica*-infected (but not *E. dispar*-infected) individuals had serum antilectin antibodies (8, 24). In Egypt it was demonstrated that 89% of patients with amebic colitis had IgG antibodies to the Gal/GalNAc lectin in their sera (2). We have also found in this study that all 16 children who were positive for *E. histolytica* by culture were positive for antilectin antibody in their serum. However, some *E. histolytica* stool infections detected by the antigen detection test in this study were seronegative. This may be due to early infection in the gut, since antigen detection is a very sensitive test and may have detected infections that are not well enough established to elicit an antibody response. Since this study was a cross-sectional sampling, we could not determine whether those stool antigen-positive but seronegative children developed an antibody response later. A controlled prospective study is currently underway in Bangladesh to understand the timing of *E. histolytica* infection and invasion and the development of immune responses in a cohort of preschool children.

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