INVASIVE AMEBIASIS: CHALLENGES IN DIAGNOSIS IN A NON-ENDEMIC COUNTRY (KUWAIT)

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Abstract. Invasive zymodemes of the enteric protozoan Entamoeba histolytica infect the large intestine and cause extra-intestinal lesions such as amebic liver abscess (ALA). The clinical manifestations of ALA are protean, particularly in patients presenting in a non-endemic, desert country such as Kuwait, and diagnosis becomes problematic. In this study, we present cases of ALA to illustrate the clinical and diagnostic challenges. For serodiagnosis of ALA, we compared the sensitivity and specificity of the indirect hemagglutination assay (IHA) with the ImmunoTab assay and an enzyme-linked immunosorbent assay (ELISA) for this geographic region. We tested sera of 110 patients with ALA, 1,224 patients suspected of having invasive amebic infection, and 50 Europeans with no travel history to an amebic-endemic area. The IHA was simple, rapid, easy to perform, and reliable (sensitivity = 99%, specificity > 95%). The performance of the IHA in detecting ALA in suspected cases was significantly better than that of the ELISA and the ImmunoTab test. Compared with the IHA, both the ELISA and ImmunoTab assay detected relatively higher numbers of false-positive cases (4.7% and 3.6%, respectively). With the availability of ultrasound and computed tomography scans, the serology correlates excellently with the clinical presentation. In chronic cases where fibrosis may be present around the abscess, the IHA has limitations, as in the follow-up of treated patients. Pitfalls in diagnosis are highlighted by discussing the differential diagnosis of ALA from bacterial hepatic abscesses and infected hydatid cysts. Most importantly, the IHA in such cases was invariably at a titer that is considered not significant.

INTRODUCTION

Infection with the intestinal protozoa Entamoeba histolytica is worldwide and approximately 500 million people each year get amebiasis, but only about 10% present with diarrhea.1,2 However, the disease potential of the organism seems to be limited to certain regions of the world where peculiar zymodemes may cause diarrhea and infection is confined to the large bowel; these organisms have been designated as E. dispar. In contrast, invasive zymodemes, regarded to be E. histolytica, have been recognized that may cause extra-intestinal infection.2 Due to the protean clinical manifestations of the latter, diagnosis is problematic and mortality may ensue, especially in vulnerable groups such as children, pregnant women, and those receiving treatment with steroids.

Developing countries have been the major casualties of amebic infection. Recently, groups at high risk for amebiasis in non-endemic and developed countries are recent immigrants, institutionalized patients, and homosexuals.3,4 The clinical importance of E. histolytica or E. dispar infection in patients with acquired immunodeficiency syndrome has yet to be determined.

New approaches for the detection of E. histolytica and E. dispar are based on antigen detection in stool and detection of E. histolytica-specific DNA by polymerase chain reaction amplification identification and differentiation.5-11 However, the result in the antigen detection test in stool is generally negative in majority of the patients with invasive amebiasis and amebic liver abscess (ALA).10 Recently, it was shown that serologic tests based on recombinant E. histolytica antigens, the serine-rich E. histolytica protein, and the 170-kD subunit of the galactose-specific adhesin may be more specific for current amebic infection than conventional tests.6,7

Kuwait is considered a non-endemic country since indigenous transmission of the E. histolytica parasite may be virtually non-existent. However, due to the large expatriate population emanating from endemic areas, the infection has been recognized in the non-indigenous population. Each year more than 50,000 immigrants from more than 50 developing countries come to work or to reside in Kuwait. However, invasive intestinal and extra-intestinal infections have also been recognized in the local Kuwaiti population, which is highly mobile traveling to endemic areas.

In this study, we present findings regarding three serologic tests we evaluated and show the indirect hemagglutination assay (IHA) to be the most suitable test for the diagnosis of ALA. Thereafter, we present our findings of the extent of the problem, the pitfalls, and the most appropriate methodology among the plethora of laboratory-based tests currently available for the diagnosis of invasive amebiasis, and present a variety of patients that present in such a diverse resident population.

PATIENTS AND METHODS

Study population. This study was carried out during the period 1996–February 2000 and included the following study groups: 1) 110 patients with ALA confirmed by ultrasonography/computed tomography (CT) and serology; 2) 50 healthy controls from developed countries with no travel history to endemic countries; 3) 1,224 patients suspected of having amebic infection who were referred by clinicians for amebiasis serology. These patients presented with various clinical symptoms: fever, weight loss, and/or pain or tender-ness in the right hypochondrium with mild hepatomegaly. Clinical suspicion of acute/invasive amebiasis was raised by either their recent travel history to endemic areas or because they were from a developing country. The informed written consent was obtained from all subjects included in the study. The study was approved by the Ethical Committee of Faculty of Medicine, Kuwait University.

Amebiasis serology. Microtiter enzyme-linked immuno-
sorbent assay (ELISA). A microtiter ELISA to detect antibodies to *E. histolytica* was done according to manufacturer's protocol (LMD Laboratories, Inc., Carlsbad, CA). Briefly, the assay was performed on diluted sera (1:64) in microtiter wells containing *E. histolytica* HK-9 soluble antigens. A titer of 1:128 and higher was suggestive of infection (manufacturer’s suggested cut-off for active or recent infection).

**Indirect hemagglutination assay.** The IHA for amebiasis serology was done according to manufacturer’s instructions (Behring Diagnostics, Marburg, Germany). Briefly, diluted patient sera were mixed with the human group O erythrocytes sensitized with soluble, purified *E. histolytica* (HK9 strain) antigen in V-shaped microtitration wells. The specific antibodies present in the serum sample cross-link the sensitized erythrocytes and the agglutinated erythrocytes settle down in the well as carpet formation. A positive IHA result was defined as a titer $\geq 1:64$ (manufacturer’s suggested cut-off for active invasive infection).

In view of high background level of seropositivity in patients from the endemic areas, the cut-off value for patients from these countries was calculated by analyzing different control groups; confirmed ALA cases, the negative control group, and patients with intestinal amebiasis. The significant titer suggestive of invasive amebiasis and/or ALA by all the three assays was calculated as $\geq 1:256$.

Predictive values of tests were calculated as the ratio of percent of true cases to total positive (true and false) cases: positive predictive value (PPV) = TP/(TP + FP), where TP = true positive and FP = false positive and negative predictive value (NPV) = TN/(TN + FN), where TN = true negative and FN = false negative. All patients positive by CT or ultrasonography with an IHA titer $\geq 1:256$ were considered true positive cases.

**Ultrasonography and computed tomography.** These procedures were done on selected patients with clinical suspicion of ALA to further define the lesion.

### RESULTS

To determine the performance of serologic assays, the patients suspected of having invasive amebiasis or ALA were screened for amebiasis serology by all three assays. The diagnosis was confirmed by ultrasonography and CT, and in some cases by fine-needle aspiration (FNA). The specimens were screened by the ELISA, ImmunoTab, and IHA tests. All 50 control specimens were negative by the three assays: IHA titer $\leq 1:64$, ELISA titer $\leq 1:128$, ImmunoTab titer $\leq 1:128$. The performance of the three assays is shown in Table 1. Our results showed that a high background titer was detected in patients from developing countries where amebiasis is endemic, especially in individuals from Pakistan, India, Sri Lanka, and Bangladesh, compared with those from non-endemic countries. One hundred fifty-four (12.6%) of the 1,224 specimens tested by the ELISA had significant titers ($\geq 1:256$) suggestive of invasive amebiasis; of these 96 (62.3%) were true positives and 58 were false positives (Table 1). The ImmunoTab assay detected significant titers in 75 (13.1%) of 574, of which 54 (72%) were confirmed to have invasive amebiasis. One hundred twelve (8.5%) of the 1,318 patients screened by the IHA test had titers $\geq 1:256$, suggesting invasive amebiasis; of these 110 (98.2%) were confirmed to have ALA (Table 1). The performance of the IHA in detecting ALA in suspected cases was significantly better than that of the ELISA and ImmunoTab test. Compared with the IHA, both the ELISA and ImmunoTab test detected relatively higher number of false-positive cases (4.7% and 3.6%, respectively). For the diagnosis of ALA, the sensitivity of the ELISA was 97.9%, its specificity was 99.8%, its PVP was 99.8%, its NPV was 99.9% (Table 1).

Table 2 shows the distribution of IHA titers in patients with ALA and in patients suspected of invasive amebiasis. All 110 patients with ALA had IHA titers ($\geq 1:256$), which is considered significant for acute/invasive amebic infection (Table 2). Ninety-seven of the 110 patients with ALA had titers $\geq 1:1,024$. In the suspected group, six patients had

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>True Pos.</th>
<th>True Neg.</th>
<th>False Pos.</th>
<th>False Neg.</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHA</td>
<td>1,318</td>
<td>110</td>
<td>1,205</td>
<td>2</td>
<td>1</td>
<td>99</td>
<td>99.8</td>
<td>98</td>
<td>99.9</td>
</tr>
<tr>
<td>IT</td>
<td>574</td>
<td>54</td>
<td>498</td>
<td>21</td>
<td>1</td>
<td>98</td>
<td>95.5</td>
<td>72</td>
<td>99.8</td>
</tr>
<tr>
<td>ELISA</td>
<td>1,224</td>
<td>96</td>
<td>1,068</td>
<td>58</td>
<td>2</td>
<td>97.9</td>
<td>94.8</td>
<td>62</td>
<td>99.8</td>
</tr>
</tbody>
</table>

*Pos. = positive; Neg. = negative; PPV = positive predictive value; NPV = negative predictive value. The cut-off titer of the IHA (1:256) for invasive amebiasis and ALA in patients from endemic areas was calculated as described in Patients and Methods.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Titer</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total no.</td>
<td>&lt;8</td>
</tr>
<tr>
<td>ALA</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>Suspected</td>
<td>1,205</td>
<td>1,012</td>
</tr>
</tbody>
</table>
The abdomen showed a large mass (9.2 cm) beneath the right dome of the diaphragm with a mixed ecogenic pattern. The CT showed a peripherally enhancing cystic lesion in the posterior segment of the right lobe of the liver and septations with increased density were also noticed.

Serology showed a titer of 1:4,096, which was considered significant for invasive amebiasis. The FNA of the lesion under ultrasonographic guidance was done and 20 ml of a viscid, chocolate, red fluid was aspirated. He was given metronidazole (800 mg, three times a day for 14 days). Prior to discharge, ultrasonography showed a smaller abscess cavity of 7.5 cm and the patient recovered completely in 8 months.

Patient 2. A 28-year-old Kuwaiti man was admitted to the Fever Hospital with high fever (40°C) and sweating. On examination, he was febrile (39°C) and had a tender liver. His white blood cell (WBC) count was 17,400/mm³ and he had no diarrhea. He had visited Mexico City six months ago. An ultrasonographic examination showed the presence of an abscess in the right lobe of the liver. Amebiasis serology showed an IHA titer of 1:1,024. He was treated with metronidazole and recovered completely.

Patient 3. A 40 year-old Italian man was admitted to the Fever Hospital with high fever (40°C) and sweating. On examination, he appeared to have toxemia and complained of pain in the right hypochondrium. He was on his way to Italy after a three-month trip to India when he disembarked at Kuwait because of his deteriorating medical condition and was admitted to the hospital. He had been on antimalarial drugs during his trip and had been eating out in restaurants. His WBC count was high (8,100/mm³) and his ESR was 93 mm/hr. His IHA titer was 1:1,024. Ultrasonography showed an abscess in the right lobe of the liver. He was treated with metronidazole and recovered completely.

Patient 4. A 54-year-old Afghan man complained of chronic pain in the right hypochondrium for the last 16 months, fever (39°C), and loss of appetite. Ultrasonography showed a mass (5.2 cm) with heterogenous echogenicity in the right lobe of the liver and seaptations with increased density were also noticed.

Serology showed a titer of 1:1,024. Computed tomography: infected cyst. The IHA titer after aspiration of the cyst was 1:2,048.

Patient 5. A 53-year-old Swedish man was admitted to the hospital with fever, weight loss, and pain in the right hypochondrium. On examination, he appeared pale and jaundiced with mild hepatomegaly. He gave a history of diarrhea four months ago. He did not recall visiting any developing countries. However, he frequently visited Chinese and Indian restaurants in Kuwait. His WBC count was 15,900/mm³ and his ESR was 120 mm/hr. Ultrasonography showed a mass (5 × 3 cm) with heterogenous echogenicity in the right lobe of liver. Computed tomography showed three focal hypo-
FIGURE 1. Computed tomography (CT) scan of the liver of patient 5, a 53-year-old Swedish man who presented with fever, pain in the right hypochondrium, and no history of travel to any endemic country. The CT shows three focal hypodense lesions in the liver: two in the right lobe and one in the caudate lobe with well-defined enhancing cyst walls. The lesion was confirmed as an amebic liver abscess by the indirect hemagglutination assay (titer $\geq 1:2,048$). The patient recovered after therapy with metronidazole and the lesions shrank after two months.

dense lesions: two in the right lobe and one in the caudate lobe with well-defined enhancing walls (Figure 1). The IHA titer for amebic serology was $1:2,048$. He was treated with metronidazole and the abscesses shrank after two months. The patient recovered slowly but without any complications.

DISCUSSION

Our primary concern relates to the fact that in a non-endemic country such as Kuwait, we are faced with expatriates harboring amebic infections. In this study, we mention at least two of these countries, India and Mexico, which have highly invasive strains and from which we had an influx of individuals. For the diagnosis of such infections, a high index of suspicion is mandatory.

It is now well recognized that in *E. histolytica* infection the antibody response varies due the individual patient response and by the type of infection. The antibody response is greatest in amebic liver abscesses, less in intestinal amebiasis, and least in asymptomatic cyst passers. Current serologic tests for detecting amebiasis, such as the IHA, are highly sensitive and specific for amebic infection. However, it is well documented that individuals with ALA may remain seropositive for amebiasis by conventional serologic tests for years following their acute infection. Furthermore, a high background level of seropositivity in endemic areas can also limit the usefulness of serologic tests in diagnosing acute or invasive amebiasis. An antigen detection test in the stool may provide an effective approach to differentiating current intestinal infection from past amebic infection; however, in $\geq 65\%$ of the ALA cases the stool was negative for antigen.

In the absence of such sensitive tests for diagnosing ALA, we used ultrasonography and CT in combination with the magnitude of the antibody level detected by the IHA to diagnose this disease. The majority ($\geq 75\%$) of our cases were from endemic countries and thus had a high background level of seropositivity that allowed us to determine a relatively higher cut-off value for such cases (IHA titer $\leq 1:2,50$). In such cases, the history of origin and travel to endemic countries is extremely useful in making a clinical suspicion of invasive amebiasis. Knowledge of the background levels in the IHA along with the availability of ultrasonography and CT allows the correlation of results of the serologic tests with the clinical presentation to obtain a meaningful diagnosis. In acute cases, this correlation with the IHA results is highly significant; however, in chronic cases in which fibrosis of the abscess wall may be present (patient 4), ultrasound or CT guided aspiration may be more useful. It is often claimed that serology is negative within the first two weeks...
of the onset of symptoms; however, because of the nature of the medical services and delayed reporting of patients to the health clinics, we do not generally see such early cases in Kuwait until the lesion develops.

Differential diagnosis of a hepatic cyst is an ongoing problem, as highlighted in Table 3. Bacterial abscesses may be caused by Streptococcus millieri or by anaerobes, as in patients 1, 2, and 5. However, in such cases the IHA test result is negative or in the low range. In this part of the world, hydatid disease, especially infected cysts (patient 6), may pose a problem in the differential diagnosis since a patient may also have a high background level of seropositivity.

Metronidazole is the drug of choice for ALA and 800 mg three times a day for 10 days has been shown to be the effective therapeutic protocol and is well tolerated by the patients. Drainage and aspiration of abscesses have been done but the criteria vary. However, there has been much discussion on the management of such patients. It is generally believed that drug treatment is the management of choice. The use of FNA with ultrasonographic and CT guidance is recommended if there is danger of imminent rupture, a slow response to chemotherapy, mixed infection, or a false-negative serology. The advantage may be that following FNA there may be an abrupt increase in the titer, indicating the amebic etiology of the abscess, as we observed in patient 4.

We have come across several cases of patients initially diagnosed as having Crohn’s disease. This is seen especially in older individuals of indigenous origin. Such patients have had past trade links with the Indian subcontinent. We have also diagnosed invasive amebiasis in these patients. Our current protocol is to ensure that before corticosteroids are administered to such a group, amebic serology is performed to rule out amebiasis.

In conclusion, we have found that the IHA test has obvious advantages over the other assays tests, especially in a non-endemic country where the number of cases are regular but limited, and it shows a good correlation with ultrasonography and CT. The drawback is that there is no correlation between clinical condition and the antibody titer. Thus, the IHA has limitations regarding the follow-up of patients in monitoring the progress of chemotherapy. In addition, we have shown that ALA is seen in the expatriate population and that invasive amebiasis, especially of the large intestine, is seen mainly in the older Kuwaiti generation who had previously visited the Indian subcontinent several times. Since the demographics of Kuwait show that there is a large population of maids/drivers and helpers from the Indian subcontinent, they may harbor the parasite and thus may be the source of indigenous transmission.

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