UTILITY OF SEROLOGY IN THE DIAGNOSIS OF AMOEBIC HEPATITIS AND HYDATID DISEASE AMONG PATIENTS WITH BENIGN HEPATIC CYSTS.

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ABSTRACT
Background: Parasites as hydatid disease and amoebic liver abscess can cause cystic lesions in the liver and their diagnosis depends on detection of IgG antibodies in the serum in combination with imaging techniques.

Aim of the work: To evaluate both sensitivity and specificity of ELISA test as a serological method for detection of IgG antibodies in patients with hydatid disease and amoebic liver abscess among patients with cystic lesions of the liver.

Methodology: A case-control study was conducted on 48 patients with cystic lesions in the liver suspicious to be hydatid cyst or amoebic liver abscess, during the period from October 2012 to December 2013. Patients involved in the study were admitted to Mansoura University Hospital at the Tropical Medicine Department and were tested for anti-hydatid and anti-amoebic IgG antibodies using ELISA technique.

Results: Thirty-one suspicious cases of hydatid disease were examined by histopathology, as a gold standard test, later; they were tested for IgG antibodies using ELISA. Histopathology detected eleven cases as hydatid disease in the liver, however, ELISA test recorded 10 positive cases and one case gave a false negative reaction, with sensitivity
and specificity 90.9% and 95% respectively. Seventeen suspicious cases of hepatic abscess were tested using IHA for anti-amoebic IgG antibodies, only 2 positive cases of amoebic liver abscess were confirmed, the two cases were also positive when using the ELISA test with sensitivity and specificity 100% and 93.3% respectively.

**Conclusion:** ELISA is a highly sensitive and specific serological technique for diagnosis of hydatid disease and amoebic liver abscess among patients with cystic lesions of the liver.

**INTRODUCTION**

Cystic lesions of the liver include wide spectrum of disorders with increased incidence nowadays due to the frequent use of abdominal imaging techniques (1). Hydatid disease and Amoebic Liver Abscess are known parasitic causes of hepatic cystic lesions (2).

Hydatid disease is a neglected zoonotic disease caused by Echinococcus granulosus, with worldwide distribution, but highest prevalence occur in Mediterranean countries including Egypt (3). As most hydatid cysts remain clinically silent and the clinical examination is unreliable, diagnosis depends on imaging techniques and serological tests, mainly antibody detection (4).

Amoebic liver abscess (ALA) is caused by protozoan parasite Entamoeba histolytica, a common parasitic infection in tropical countries (5). Detection of trophozoites using microscopic examination of liver aspirate, though confirmatory of ALA, is quite insensitive (6). Diagnosis of ALA is frequently conducted using serum anti-amoebic IgG antibodies (7).

The aim of this work was to evaluate the sensitivity and specificity of ELISA test for detection of hydatid disease in the liver and ALA among patients with hepatic cystic lesions.

**SUBJECTS, MATERIALS AND METHODS**

This study was conducted, during the period from October 2012 to December 2013, on 48 patients with benign hepatic cysts; suspected to be parasitic in origin who were admitted to Mansoura University Hospital at the Tropical Medicine Department. Patients were subjected
to complete history taking, clinical examination and abdominal ultrasonography to evaluate hepatic cysts which could be hydatid disease or amoebic liver abscess. A blood sample (5ml) was taken from all patients, left to clot and serum was obtained by centrifugation then stored at -20°C until use for detection of IgG antibodies against E. granulosus and E. histolytica using Echinococcus ELISA (Cat# 8202-35, Diagnostic Automation, USA) and Entamoeba histolytica Amebiasis ELISA (Cat# 8201-35, Diagnostic Automation, USA). Briefly, serum samples were diluted using dilution buffer supplied with the kit and incubated with the coated plates for 10 min at room temperature. After washing, an enzyme conjugate was added for 5 min, followed by washing and finally, chromogen was added and the reaction was stopped using stop buffer. Negative and positive controls included in the kit were tested to check validity of the kit. In addition, healthy controls, 11 persons, were tested to check for false results. Moreover, 7 patients with other parasitic infections (3 schistosomiasis mansoni, 2 toxoplasmosis and 2 giardiasis) were used to test for cross reactivity.

**Ethical consideration**

This study was approved by medical ethics committee of Faculty of Medicine, Mansoura University, Egypt. Informed and written consents were obtained from all participants in the study.

**Statistical analysis**

Data were statistically analyzed using EXCEL to obtain analytical statistics [sensitivity, specificity, diagnostic accuracy, positive predictive values (PPV) and negative predictive values (NPV)] of each assay.

**RESULTS**

Thirty-one cases of clinically and radiologically suspicious hydatid disease in the liver were examined by ultrasonography, CT (Fig. 1), IHA in addition to histopathology. Eleven cases were proved to be positive for hydatid disease by histopathology (Fig. 2) and 20 cases were free of the disease. All the 31 case were tested for anti-hydatid IgG antibodies by ELISA technique. From the 11 confirmed cases, only one case gave false negative by ELISA test (Table 1). Sensitivity and specificity of ELISA test for diagnosis of hydatid liver disease were calculated as shown in (Table 2). Serum samples...
from controls used in the study were tested using ELISA test and only one sample from schistosomiasis patient had cross-reactivity with the kit (Table 3). Seventeen suspicious cases of hepatic abscess were tested for liver amoebic infection using IHA and ELISA kit and in both, two cases only were recorded as positive for amoebic liver abscess using IHA and ELISA, however suspected case recorded negative by IHA but was positive (false positive) with ELISA test (Table 4). The sensitivity and specificity of ELISA test were 100% and 93.3% respectively (Table 5). From the controls, only one case of Giardia showed cross-reactivity with the tested ELISA kit (Table 6).

Table 1. Suspected cases of hydatid disease and their reaction with ELISA test.

<table>
<thead>
<tr>
<th></th>
<th>Patients with disease</th>
<th>Patients without disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA test positive</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>ELISA test negative</td>
<td>1</td>
<td>19</td>
</tr>
</tbody>
</table>

Table 2. Sensitivity and specificity of ELISA test for hydatid disease patients

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Diagnostic Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>90.9%</td>
<td>95%</td>
<td>90.9</td>
<td>95</td>
<td>93.5</td>
</tr>
</tbody>
</table>
Table 3. Detection of antihydatid antibodies in hydatid disease patients and controls using ELISA test

| Subject groups             | Positive results with ELISA (%) |
|---------------------------|--------------------------------|---------|
| Confirmed cases (n = 11)  | 10 (90.9)                      |         |
| Disease controls (n = 7)  | 1 (14.3)                       |         |
| Healthy controls (n = 11) | 0 (0)                          |         |

Table 4. Suspicious cases of amoebic liver abscess and their reaction with ELISA

<table>
<thead>
<tr>
<th>ELISA test positive</th>
<th>Patients with disease</th>
<th>Patients without disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA test negative</td>
<td>0</td>
<td>14</td>
</tr>
</tbody>
</table>

Table 5. Sensitivity and specificity of ELISA test for diagnosis of ALA

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Diagnostic Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>100%</td>
<td>93.3%</td>
<td>66.7</td>
<td>100</td>
<td>94.1</td>
</tr>
</tbody>
</table>

Table 6. Detection of anti-amoebic antibodies in amoebic liver abscess patients and control

| Subject groups             | Positive results with ELISA (%) |
|---------------------------|--------------------------------|---------|
| Confirmed cases (n = 11)  | 10 (90.9)                      |         |
| Disease controls (n = 7)  | 1 (14.3)                       |         |
| Healthy controls (n = 11) | 0 (0)                          |         |
**Figure 1:** Imaging of the liver:

(A) Ultrasonography of the liver showing cystic lesion
(B) Post-contrast CT for the liver showing right lobe cystic lesion with mural calcification (white arrow).

**Figure 2:** Histopathological feature of Cystic Echinococcosis. Open arrow refers to cyst wall and arrow heads pointed to protoscolices.

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DISCUSSION

Diagnosis of liver CE is usually accidental, associated with an abdominal sonography performed for other clinical reasons. Presence of symptoms suggestive of CE in a person, who is in contact with dogs, supports the suspicion of the disease. Radiological imaging, especially abdominal ultrasonography, is considered the gold standard for detecting the number, site, size and vitality of the hydatid cyst (8) and it is also important to determine the treatment strategy. But, ultrasonography is not always able to differentiate hydatid cysts from other space-occupying lesions, as tumors or abscesses, so additional techniques are required to confirm the radiological diagnosis. Immunodiagnosis is an important technique to validate the imaging diagnosis and can be used for treatment follow up even if there is undetectable immune response (9). The gold standard laboratory tests for human echinococcosis are currently based on standard ELISA or immunoblot using E. granulosus hydatid cyst fluid-derived antigen B (10).

In this study, plates coated with Echinococcus cyst wall antigen for diagnosis of liver CE were used. Although the test was qualitative, it was able to detect CE in 90.9% of the cases. The obtained sensitivity is nearly similar to those obtained from the most commonly diagnostic tests used for diagnosis of CE based on hydatid fluid antigens, which have relatively high sensitivity for hepatic cysts ranging from 85% to 95% (11). More recent, the recombinant form of AgB, a protein derived from cyst fluid, has a sensitivity of 97.1%, but cross reaction with other cestodes were recorded (12) resulting in specificity of 62.7%, which is very low compared to our study (95%).

In our study, only one positive case for hepatic CE was negative by the tested kit. This case had a single small cyst (2 cm), this data suggested that cyst size could be
one of the limitations of this kit and only patients cysts bigger than 2 cm are detected using this kit.

One of the important problems encountered was the observation of false positive cases caused by cross-reaction with other helminthic infections. We have recorded only one false positive case due to schistosomiasis mansoni, as Schistosoma probably shares epitopes with cestodes, but we are expecting more cases especially those infected with other cestodes as Taenia species and more parasite-positive controls should be tested for comprehensive evaluation of this kit. CE, especially in the liver, is characterized by the triggering of an intense humoral response with rising titre of antibodies (9). Such response is encouraging for development of novel CE serodiagnostic assays. Although we have plenty of these tests, the used antigens do not provide an appropriate level of sensitivity and specificity. Although the initial evaluation of the new ELISA kit used in this study was promising, since antigens from the cyst wall were used, a single defined recombinant E. granulosus antigen is required urgently to ensure early diagnosis and limited or even no cross reactivity.

Conventional diagnosis of ALA is confirmed by finding the E. histolytica trophozoites in liver pus aspirate obtained via ultrasound guided percutaneous aspiration biopsy, but the parasites are often absent as most of them are located at the margin on the periphery of the abscess (5). Therefore, serodiagnosis is widely adopted for diagnosis of ALA, detecting either amoebic antigens or antibodies from serum samples. However, for antigen detection, available tests showed lower sensitivities ranging from 8.5% to 50% (13,14). Hepatic amoebiasis raises a strong humoral immune response especially immunoglobulin G (15) and there are a variety of serological assays used for diagnosis of ALA based on detecting circulating antibodies such as IHA, Latex agglutination, indirect immunofluorescence, counter-immunoelectrophoresis, gel diffusion, complement fixation and ELISA; IHA and ELISA are still the preferred choices (16).

ELISA is commonly used as a routine diagnostic assay in diagnosis of ALA because it can be developed
in-house use based on different amoebic antigen preparations such as crude soluble antigen (CSA), excretory-secretory antigens, plasma membrane antigens, purified antigenic proteins or recombinant proteins. The CSA-based ELISA technique has been used in routine diagnosis of ALA with reported 100% sensitivity and more than 90% specificity (17). In our study, testing a novel ELISA kit utilizing crude amoebic antigens showed 100% sensitivity; this result is in agreement with the previously recorded results using CSA and better than those recorded when using recombinant proteins (18,19). However, cross-reactivity was reported with serum from patient complaining of Giardia infection with 93.3% specificity. False positive diagnosis of ALA reported in our study could be attributed to variability in the antigen preparations since this antigens prepared from amoeba maintained in the culture and the proteins used for diagnosis may vary from patch to patch. Although several proteins have been evaluated by ELISA technique for diagnosis of ALA, they were less sensitive and immunoreactivity have been reported with amoebic dysentery and even asymptomatic cases (20). In the current study, this new test showed promising diagnostic validity.

In conclusion, in this work, we have detected that ELISA could be useful for diagnosis of hepatic CE and ALA; however a future study with more patients is needed to assess the validity of kits used in this study.

REFERENCES


linked immunosorbent assay. Parasitol Res 97, 209-212.