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Reliability of Immunodiagnostic Tests for Diagnosis of Liver Alveolar Echinococcosis

KEYWORDS: Alveolar echinococcosis, Echinococcus multilocularis, Serodiagnostic test

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ABSTRACT *Background and Aim: Liver alveolar echinococcosis is a rare parasitic zoonosis, is caused by Echinococcus multilocularis and is a very rare and severe disease. Our aim is to investigate the accuracy of diagnosis of Alveolar Echinococcosis (AE) via different serological tests, with an emphasis on pats who had liver resections. Methods: We investigated 39 patients who were diagnosed with liver AE. Group 1 (n=16) inoperable patients, Group 2 (n=11) complete resection of all parasitic mass, Group 3 (n=12) partial resection in which a small remnant was left on a vital structure. We investigated three different markers Em2plus, Em12, and Em16-18. Results: We found that sensitivity for serologic diagnosis of AE was 88% (Em2plus), 94% (Em16-18), and 69% (Em12) in group 1, 55% (Em2plus), 27% (Em16-18) and 18% (Em12) in group 2, 75% (Em2plus), 100% (Em16-18), 75% (Em12) in group 3 respectively. Conclusions: In conclusion, the serologic tests are practical and cost-effective tools for use in the diagnosis of the AE. In our study the 16-18 bands observed in with the western blot method, are determined to be the most reliable test in the diagnosis of AE for groups 1 and 3. For group 2, Em12 and Em16-18 may both be used as markers for patient follow-up tests.*

INTRODUCTION

Alveolar echinococcosis (AE) is a rare parasitic disease that is caused by the larva of Echinococcous multiocularis (Em) [1-5]. In humans, this zoonotic infection is characterized by its predominantly hepatic involvement, extensive local tissue invasion and destruction, and ability to form distant metastases, causing it to be compared to a malignant process [6]. Infection is often undetected for many years of parasite persistence, and typically found incidentally during imaging [7]. Epidemiological and clinical data, the high prevalence of EM in foxes in endemic areas but the very low incidence of AE in the human population, suggest that exposure to Em does not progress to clinical disease in all cases because many subjects present abortive and spontaneously healed lesions after infection [8-10]. Treatment of AE requires surgical intervention, if possible radical, combined with chemotherapy using benzimidazole carbamate derivatives [11, 12]. However, the disease amenable to curative resection in only 20-30 percent of patients at the time of diagnosis [13].

Diagnosis of AE is primarily based on imaging techniques including ultrasonography, computed tomography and magnetic resonance imaging [14] in addition to clinical criteria [15, 16]. However, imaging techniques are relatively complex, do not always offer a good prospect for early diagnosis, and produce data that are sometimes difficult to interpret, being often confused with those from abscesses and neoplasms [17]. Moreover, these techniques are too expensive and inaccessible in most areas where AE is endemic. Immunodiagnosis of alveolar hydatid disease is useful, effective, and more reliable than the diagnosis of cystic hydatid disease. Serological methods using ELISA and Western blot technology are important not only for confirmation of AE cases, but also for epidemiological studies in endemic areas [18]. Firstly, Gottstein et al, prepared an antigen fraction (Em2) from alveococcus tissue using affinity chromatography [2]. After, the immunodiagnosis of AE by western blot, Ito et al. identified two specific antigenic components of EM protoscolex, designated Em18 and Em16, which were detectable exclusively with sera from active AE patients [19].

The objective of our study was to comparatively evaluate serologic tests as a diagnostic and disease marker for patient follow-up of AE with and without surgery. Specifically, we tested three commonly employed tests: ELISA (Em-2plus), Western blot (Em16-18, Em 12).

MATERIALS AND METHODS

A total of 39 patients who were diagnosed and treated for AE localized to the liver were included in this multicentric study from 1999-2010. The patient records were reviewed, and the data results were recorded on a standard form. This included age, sex, location, surgical methods and mortality. Postoperative mortality was defined as death occurring in the time prior to discharge from the hospital. Pathological diagnoses were carried out on all patients. The diagnosis of AE was based on history, imaging techniques and confirmed by histopathological examination. The study protocol was approved by the Ethics Committee at Istanbul Faculty of Istanbul University.

Patients were divided into three groups according to the

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therapeutic procedure: Group 1 (n=16) included patients who were diagnosed but considered inoperable due to advanced disease; Group 2 (n=11), patients who had curative resection (R0:complete resection of all parasitic mass); Group 3 (n=12), patients who had near total resection (R1:a resection in which a small remnant was left on a vital structure) or debulking resection of hepatic AE. (Serum samples were taken after the diagnosis in patients with pathological and imaging studies.) From all of the patients venous blood samples were taken for serologic diagnosis of AE. Serum samples were used for assessing diagnostic sensitivities of ELISA and Western blot methods. We used two different commercial tests for the serodiagnosis of AE in humans (Em2plus Elisa; Bordier Affinity Products, Switzerland) and Echinococcus Western blot IgG (LDBIO Diagnostics, France). We investigated three different markers including Em2plus, Em16-18 and Em12.

With all patients, albendazole 7.5mg/kg twice a day was administered initially with dosages in the long term modified as necessary based on the patient and treatment progress.

ELISA; Border Affinity Products SA Company's ELISA kits were used for detecting antibodies against E multiocularis Em2plus antigen, AE. The according to manufacturer's instructions. E multiocularis Em2plus antigen sensitized microtitration plates were used. Presence of IgG antibody in serum was detected with protein anti-human IgG-alkali phosphatase conjugate (Sera were tested according to the procedure step by step. Bubbles are trapped in microplate reading was before). Results were evaluated via spectroscopy at 405nm. Substract value of the no serum blank from all measured values. The test is valid if the following criteria are met: absorbance (A) of positive control >1000, A of negative control>10% of A of positive control, A of blank against air >0.350. The antibody concentration of the weak positive (Cut off) serum has been set to discriminate optimally between sera of cases of AE and normal human sera. A sample with lower than the weak positive control (Cut Off) serum has a non-significant antibody concentration against Echinococcus multilocularis Em2plus antigen, it is therefore serologically negative. A sample with an absorbance higher than the weak(Cut off) control serum is serologically positive. The test employed has a reported %93 sensitivity and %98 specificity.

Western Blot; Western Blot kit from LDBio Diagnostic, France as, which contain EM antigens obtained from larval extract decomposed to electrophoresis bands and transferred to nitrocellulose membranes with electro blotting. Echinococcus-specific IgG, if present in the serum, will bind to antigens on the strips. The strip is then incubated with an alkaline phosphatase-anti human IgG conjugate. Following the wash step, the whole complex is then detected by addition of substrate which precipitates as a dark blue-purple colour. After the distilled water, Echinococcus-specific IgG, if present in the serum sample will appear on the strips as violet coloured bands. The presence on the strip of the 7 and/or the 26-28 kDa band(s) is indicative of presence of Echinococcus-specific IgG in the serum sample(20). WB bands including five typical patterns (P1-P5) for differential diagnosis between E.granulosus and E.multilocularis. P1 pattern is including only 7 kDa band for E.granulosus. P2 pattern is including 7 kDa and large fuzzy band 16-18 kDa for E. granulosus. But P3 is including 26-28 kDa and both narrow bands 16 and/or 18 kDa bands for E. multilocularis. Besides P3 patters can also be present is most of the other bands (7,12,15,17,20 kDa). P4 pattern is only 26-28 kDa and no intermediate band, P5 is including 7 and 26-28 kDa band and no other intermediate band. P1, P2 and P3 are species specific and differentiate E. granulosus from E. multilocularis. P4 and P5, cannot distinguish between the 2 species.

Statistical analysis; normal inspection was done with Shapiro wilk test by drawing a histogram bar. Data were presented with a minimum maximum median frequency and with percentages. Measurement variables between the groups were compared Kruskal- Wallis one way analysis of variance, categorical data with Yates- corrected chi-squared test and Fisher exact probability test. P< 0.05 was regarded as the significance limit and as two ways. Analyses were done with SPSS 21.0 programme.

RESULTS

Patient demographics and follow-up results are outlined in Table 1. There were no postoperative surgery related deaths in either the debulking or curative surgery groups. We found that sensitivities for serologic diagnosis of AE were 88% (Em2plus), 94% (Em16-18), and 69% (Em12) in Group 1, 55% (Em2plus), 27% (Em16-18) and 18% (Em12) in Group 2 , 75% (Em2plus), 100% (Em16-18), 75% (Em12) in Group 3 respectively. The serological test of Em16-18 was found most sensitive test in Group 1 and Group 3 (Table 2).

Age and sex were distributed as homogenous between the groups (p> 0.05). Fisher exact probability test were distributed similarly in Em2 plus between 3 groups.

Between 3 groups, Em 16-18 value was found to be rather low in group 2 compared to the ones in group 1 and group 3. Em12 value was found to be low in group 2 compared to the one in group 1 and group 3.

Table 1. Patient characteristics and follow-up period (months). Kruskal Wallis one way analysis variance

Patient Characteristics and Follow-up results	Group 1 (n=16)	Group 2 (n=11)	Group 3 (n=12)	Overall (n=39)	P
Median Age (range)	34.9 (22-58)	35.2 (24-51)	34.3 (14-47)	34.7 (14-58)	P>0.05
Male/Female	7/9	6/5	8/4	21/18	P>0.05
Mortality	-	-	-	-	
Median Follow-up duration (range)	62 (18-132)	64.7 (18-144)	58.3 (16-88)	61.6 (16-144)	

Table 2. Diagnostic sensitivities for the different antigens and patients with and without surgery of liver AE. Group 1: inoperable patients, group 2: performed R0 resection, group 3: performed R1 resection. Fisher exact probability test.

(n %)	Group 1 (n=16)	Group 2 (n=11)	Group 3 (n=12)	P
Em 2plus	%88	%94	%69	p=0.13
Em 16-18	%55	%27	%18	p<0.001
Em 12	%75	%100	%75	p=0.015

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DISCUSSION

More than 98% of primary infections in human AE cases appear in the liver, with long asymptomatic periods (5 to 15 years). By the time signs and symptoms become evident, the disease process may be so advanced that the disease is difficult to treat. Therefore early diagnosis and treatment are crucial for the reduction of morbidity and mortality [20]. Using molecular and immunological techniques many researchers have attempted to identify EM-specific antigens and showed the usefulness of recombinant antigens for serodiagnosis [21]. Serological methods using ELISA and western blot technology are important not only for confirmation of AE cases, but also for epidemiological studies. However, clinical and diagnostic studies of AE are limited by the rarity of the disease and insufficient numbers of patients within the study period. Therefore in this study, we comparatively evaluated three commercially available serologic test kits in diagnosis and follow-up of patients. The groups tested were patients with AE with surgery (especially R0 resection), AE with R1 resection and without surgery. We used two different serologic tests that including assays by ELISA (Em2plus), Western Blot Em16, Em18 and Em12. Currently, reports in the literature describe a total of 14 different ELISA tests that have been used to diagnose AE [22, 23]. Em2 and 2/3-10 have been used simultaneously in an ELISA kit (known as Em2plus ELISA), showing excellent specificity and only a minor loss in diagnostic sensitivity [24]. Frosch and others characterized a full length mRNA of 65-kDa protein from EM protoscolices, and showed that the expressed antigen, designated Em10, had potential for use in diagnosis of AE [25]. In an attempt to improve the immunodiagnosis of AE by western blot, Ito et al. identified two specific antigenic components of Em protoscolex, designated Em18 and Em16, which were detectable exclusively with sera from active AE patients [26].

Results indicate that Em 16-18 was the most sensitive test for groups 1 and 3. For Group 2, i.e. in patients with R0 resections, Em2plus was the most sensitive test for determination of cure (55%), and the least sensitive was Em12 (18%) with EM16-18 at 27%. Further analysis of Group 2 indicated Em12 test results were negative, after 24 months, whereas for Em 16-18 this occurred after 36 months .These results indicate that Em12 and Em16-18 tests are useful to evaluate the efficacy of R0 surgery (Group2). In group 3, where the disease is palliatively operated, and in group 1 where surgery was not possible, Em16-18 has been identified as the most sensitive test 18 (100%, 94% sensitivity respectively). These results are in general concordance with the literature, where Em18, an 18kDa antigen from protoscolices of AE was reported as being a highly species-specific (96.8%) and sensitive (97%) [27]. In another study, the diagnostic value of Em18 was tested with ELISA and immunblotting and the sensitivity, performed with both techniques on AE patients, varied from 87.1% to 100%, respectively [28].

In conclusion, the serological diagnostic tests are practical and cost-effective tools the diagnosis of the AE. In our study western blot method employing the Em16-18 bands shown was determined to be the most reliable test in the diagnosis of AE for groups 1 and 3. For group 2, it was concluded that either Em12 or Em16-18 could be used as a marker for cure.

CONFLICTS OF INTEREST

None.

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