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Original Research Article

Diagnosis Efficacy of Serological Test in Echinococcosis Cysts: A Retrospective Study

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Abstract

The diagnosis of cystic echinococcosis (CE) is based on imaging, but it is recommended to use serological test to support imaging in inconclusive cases. We performed a retrospective analysis of serology results of patients with CE lesions, seen from January 2016 to March 2020, to evaluate the contribution of the serology in the diagnosis accuracy and sensitivity of CE. Sera from eligible patients, including 86 patients with probable CE lesion, were used, with 2 commercial seroassays (NovaLisa^{TM} *Echinococcus* IgG; NovaTec Immunodiagnostica, Germany and Western Blot; ECHINOCOCCUS Western Blot (WB) IgG from LDBIO diagnostics). Sensitivity of seroassays to liver cyst wasn't significantly higher than lung cyst, but a statically significant correlation has been found between positive CE serology and complicated cysts (82% versus 55% p<0.001). Sensitivity and diagnostic accuracy of ELISA test combined to Western blot (WB) were higher than those obtained with ELISA test alone (65% versus 82% p= 0,001). Combining a first level seroassays as ELISA test with a high specific test as WB provide the best diagnosis accuracy for CE.

Keywords: Cystic-echinococcosis-serology- diagnostic.

INTRODUCTION

Human cystic echinococcosis (CE) is a chronic infection due to the larva of *Echinococcus granulosus sensu lato* (s.l). [1]. The infection is prevalent worldwide where livestock breeding is practiced [2], and humans are accidental intermediate hosts, acquiring the infection through the ingestion of infective parasitic eggs [3].

Most infected people, especially those harboring abdominal CE, are asymptomatic or paucisymptomatic, and often, echinococcal cysts are diagnosed during imaging examinations performed for other reasons [3].

CE diagnosis is based on imaging tools (abdominal ultrasound, chest X-rays, computed tomography [CT] scan or *magnetic resonance* (*MR*)) [4]. However, specific expertise in US and on the recognition of the pathognomonic features of CE cysts on imaging are needed for a correct diagnosis and management of the infection to avoid unnecessary or inappropriate treatment, with the consequent risks and costs.

The differential diagnosis of CE is broad, ranging from harmless biliary cysts to cancer, and this poses serious problems in endemic, resource-poor settings where expertise in US and availability of invasive options for differential diagnosis are scant [5].

For that reason, In inconclusive cases, the WHO-Informal Working Group on Echinococcosis (WHO-IWGE) recommends assessing specific serum antibodies [6, 7].

Currently serological available tests lack standardization and present unsatisfactory sensitivity and specificity [5] and the results must be interpreted in the light of imaging characteristics [8].

The aim of this work was to evaluate the contribution of serology in the diagnostic accuracy in endemic zone with cystic Echinococcosis.

PATIENTS AND METHODS

The study was a retrospective analysis of data from routine diagnostic tests. clinical and serological data of eligible patients diagnosed between January 2016 and March 2020 were analyzed.

Patients with CE were diagnosed using US or CT-scan, performed by experienced physicians.

The final diagnosis was confirmed by surgery, histology, and/or parasitology for confirmed CE and by suggestive imaging findings and positive IB Echinococcus serology for probable CE according to the World Health Organization consensus definition of probable CE [6].

In the study, we used a qualitative ELISA test: NovaLisa™ *Echinococcus* IgG (NovaTec Immunodiagnostica, Germany), coated with *Echinococcus* crude antigen. And western Blot test: ECHINOCOCCUS Western Blot (WB) IgG from LDBIO diagnostics, France).

All methods were used according to the manufacturer's recommendations. For Elisa test, the cut-off is the mean absorbance value of the cut-off control determinations. Samples are considered positive or negative if the absorbance is 10% higher or lower, respectively than the cut-off absorbance. If the absorbance is in the grey zone, we repeated the test within 2 or 4 weeks with a fresh sample, as the manufacturer recommends. For WB, The diagnosis was retained in front of the presence of 2 bands, 16 and 18 KDa.

The criteria for inclusion were as follows: 1) the presence of CE or suspect CE lesion(s) visualized on imaging, 2) having been tested with serology assay.

Parameters are reported with a 95% confidence interval (CI) and a statistical significance level fixed at 0.05. The IBM-SPSS software version 19 was used for the statistical analysis.

Before analysis, all data were pseudonymized; no ethical clearance was required for this work.

RESULTS

A total of 86 serums were examined, and 79 patients were diagnosed with CE on the basis of radiology or histopathology.

The median age of our patients is 36 years (9–71 years). The distribution by sex was 35 women (40%) and 51 men (59%).

Of patients with CE, 59% (n=47) had liver cysts; 35% (n=28) had lung cysts and 2% (n=4) had multifocal cyst. Some disease progression complications of CE were found in 37%(n=28) of the patients.

The sensitivity of the ELISA test for CE was 65%, versus a sensitivity of up to 82% of the couple ELISA+ Western blot (p=0.001), and the specificity was 100%.

The sensitivity of seroassays for liver cyst was slightly higher than their sensitivity for lung cysts, but no significant association was found.

In other hand, the sensitivity of seroassays for complicated cysts (rupture or infection) is up to 82% versus 55% for non-complicated cyst (p<0.001).

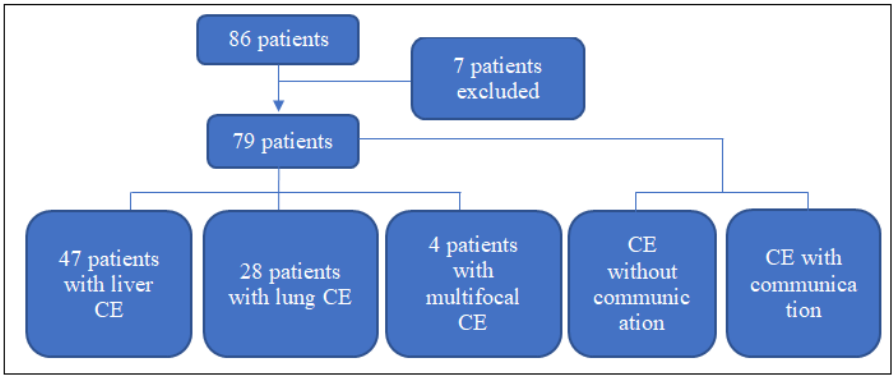

Figure 1

Table 1: Sensitivity of immunoassays for echinococcal cyst

	SENSITIVITY (CI 95%)		
	LIVER CE	LUNG CE	*P*
ELISA	0,68 (n=32)	0,60 (n=17)	NS
ELISA+WB	0,87 (n=41)	0,78 (n=22)	0.041

Table 2: Sensitivity of immunoassays for echinococcal cyst

	CE COMPLICATED	CE NON COMPLICATED	*P*
SENSITIVITY (CI 95%)	0,82(n=23)	0,55(n=26)	<0.001

DISCUSSION

Serology is used to support imaging in doubtful cases. However, currently available serology tests are not standardized, and their performances varies greatly [9]. Several factors, including CE cyst location, stage, size, number, previous treatments, the presence of any cyst complication, and characteristics of hosts such as age and immune response, influence the outcome of serology tests and induce a lot of false negatives [8].

Consequently, the interpretation of seroassays is very challenging, especially that no consensus algorithm is available.

The range of diagnostic accuracy with serologic tests in hydatid cysts is 43 to 94% [10]. The ELISA test coated with native antigen gives moderate sensitivity values with an acceptable range of specificity. Otherwise, by using recombinant antigen, the ELISA test improves sensitivity, but at the expense of specificity [5]. In accordance with literature, we found an overall sensitivity of 65%, regardless of the location and integrity of the cyst.

Using the same ELISA kit: NovaLisa^{TM} *Echinococcus* IgG (NovaTec Immunodiagnostica, Germany), Merve Aydin and colleagues found a very low sensitivity of 35,5% [11], Yener Aydin and colleagues found 57% in a study of only lung cyst [12], Ahmad and al., 66% [13], and Feckov and al. 77,8% [14].

Lung cysts seem likely to induce a weaker immune response than liver cysts, as well as all other locations [15], This fact was not clearly demonstrated in our data, which could be attributed to the small size of our population or the fact that two-thirds of the lung cysts were complicated by rupture or infection.

Combining several serological tests in routine practice provides increased sensitivity of the serodiagnosis of CE, which compensates for negative results, as demonstrated in several studies evaluating the efficacy of various serological test combinations in human CE diagnosis [16, 17].

Authors suggested that the best diagnostic accuracy was obtained using one or two first-line tests, followed by a highly specific third test as WB [3, 6], similarly to what can be derived from our data, or using WB as a single test, especially for liver CE [10]. However, WB is expensive, requires specifically trained personnel for its interpretation, and is often used only as a confirmatory second-level test.

In our study, combining ELISA and WB provided the best diagnostic accuracy, with a sensitivity of up to 87% versus 67% when the ELISA test was used alone.

Our data show a very high specificity of the serology tests applied. This result was expected because of the inclusion criteria and the retrospective character of the study. Similar to our results, many studies obtained very high specificities because of the nature of control groups that chose patients with lesions that might be differential diagnosis with CE [18].

Generally, false positivity occurs because of cross-reactivity with other parasites such as Echinococcus *multilocularis*, visceral leishmaniasis, distomatosis, taeniasis, toxocariasis and *Taenia saginata* [1]. unfortunately, our control group was not sufficiently diversified to better verify the specificity of our tests, but a lot of authors confirmed that WB is the best test to apply for the differential diagnosis of CE [3, 15], but it must be interpreted with concordant imaging [15].

This study has several limitations. First is the retrospective design, and the absence of a control group more diversified with patients having several parasitic infections, in addition to patients having cysts and tumors that may enter differential diagnosis with CE. And finally, the collected data should provide information about the number of cysts, their locations and staging of cysts, and the serological status of patients vis-à-vis the various parasitic infections that may interfere with results.

In conclusion, the diagnostic approach for CE should involve a combination of imaging techniques and serological tests. The ELISA test confirmed by WB is highly sensitive and seems to provide the best diagnostic accuracy for CE. A serological test shouldn't be used to exclude the diagnosis of CE, especially in endemic area with CE.

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