

Development of a New ELISA Kit for the Diagnosis of Hydatidosis in Humans

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Key Words: Hydatidosis, Echinococcosis, ELISA kit, CCIE crude antigen

ABSTRACT

Cystic hydatid disease (Hydatidosis) is the most serious tape-worm infection prevalent in the cattle and sheep raising area of the world. Hydatidosis in man (as an accidental host) caused by infection with the ova containing larval stage of *Echinococcus spp.* In the last decade different techniques have been employed for the serological diagnosis of hydatid disease, such as IHA, IFA, ELISA, CCLE (Counter Current Immuno Electrophoresis). The immunologic techniques used in this study were ELISA and CCIE. Since whole hydatid cyst fluid has been used as a source of antigen for serodiagnosis of hydatidosis. The result of examination of 30 patients that were surgically and pathologically proven to have hydatidosis was presented here. By appointing 1:100 serum titer and using the crude antigen (concentration: 6 µg/ml), sensitivity and specificity of the Elisa test were reported to be 93.3% and 96.6%, respectively. The Elisa was compared with CCLE in this study; it was found to have sensitivity and specificity 90% and 100%, respectively. Finally, the result of our study showed that the ELISA kit designed in our study is easy to perform, not expensive, safe, and simple with good sensitivity and specificity.

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INTRODUCTION

Echinococcosis (Hydatidosis) is a human disease caused by the larval (meta cestode) from *Echinococcus spp.* which live in the gut of the dog, wild canines and other carnivorous animals representing the definitive hosts, or involve as intermediate hosts in both domestic and wild animals (1,7).

Human becomes accidental intermediate hosts by ingesting taenia eggs. The main species pathogenic for man are *E. granulosus* causing cystic echinococcosis with world wide distribution and endemic in sheep and cattle breeding countries, and *E. multilocularis* causing alveolar echinococcosis with preferential distribution in the northern hemisphere (1,11).

The diagnosis of hydatidosis is based primarily on radiologic and serologic methods, which is needed before, a decision to perform surgery. Sensitive and reliable serologic techniques are necessary to confirm the diagnosis (9,10).

In this study we report the diagnosis of hydatid cases by Enzyme Linked Immunosorbent Assay (ELISA) for 30 patient with surgically proven hydatidosis. We have compared two serologic methods to find the sensitivity and specificity of this assay, the performance of two recently developed methods; ELISA and counter current Immunoelectrophoresis (CCIE) together.

MATERIALS AND METHODS

Patients and serum samples: Blood were collected from three main groups of men (10). The first group of sera was

washing buffer and peroxidase conjugated (lyophilized HRP-labeled sheep antihuman immunoglobulin) was added to the wells at optimal dilution in PBS and incubated at room temperature for 1h.

TMB substrate (tetramethyl benzidine citric is combined with urea peroxide solution) was added the plates were incubated at 20° for 30 min. The reaction was stopped by adding 4 mol/m sulfuric acid to each well immediately After incubation. Absorbance values were measured at 450 nm using a automatic microplate reader.

RESULTS

Of the 30 patients (13 women and 17 men) in this study, 15 had hydatid cyst in the liver, 9 had lung cyst and six had cyst in the other organs includes spleen, kidney, bone, ... (Table 1).

The presence of antibodies against *Echinococcus granulosus* in sera from the 30 patients was investigated by ELISA. In this study only 2 of 30 patients gave negative result, and 28 had positive reactions. Only one positive reaction were observed among 30 healthy volunteers (Table 2).

In the patient with CE, 93.3% positive reactions were recorded for the detection of specific IgG antibodies. The ELISA WHF gave identical result by this method. In this study the sensitivity and specificity of the ELISA were reported to 93.3% and 96.6%, respectively (Table 2). 28 of 30 sera from patients were interpreted as positive. In contrast, all sera from cases with proven

tested by CCIE. Comparative sensitivity of (93.3%) and higher specificity of CCIE than Elisa

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The sera was collected from non-Echinococcus human sera samples from person with other disease. In addition to these 30 samples a further 30 serum sample were used as control negatives and were collected from healthy donors.

Antigen Preparation

Whole hydatid fluid was used for ELISA and CCIE. This antigen was isolated from fertile live sheep hydatid cysts and processed according to the technique described by Capron and others (1).

Serological Techniques

Two methods have been previously used to detect hydatid antibodies; ELISA WHF and CCIE.

Recognition of hydatid antigen by sera was done by Elisa according to the following modifications:

Antigens were coated with 200 microliter per well at a concentration of 6µg/ml, diluted in bicarbonate buffer pH 9.6 (BCB) and incubated over night at 4°. Wells were washed four time in washing buffer (phosphat buffer saline tween-20). Human serum samples were incubated for 1h at room temperature after dilution with PBS tween-20 (1:100) then washed four time with

In ten cases, cross-reaction was observed when sera from patients with different parasitic infection were analysed for anti echinococcus IgG antibody (Table 3). The highest cross-reactivity was found in sera from patients with fasciola (7 of 15). Also in one patient with toxocariasis (n=4), fasciola (7 of 15). Also in one patient with toxoplasmosis (n=5) and liver cirrhosis (n=6) positive reaction was observed.

DISCUSSION

Since *E. granulosus* is the most prevalent agent of hydatidosis in human, all the attempts are being focused to obtain a proper antigen for serological test.

Nowadays there are various methods for detection of hydatid cysts like Radiography, ultrasound, etc.

However, to perform these tests, they have some limitations. Since, fast detection of the cases in the endemic areas is very important, in order to prevent further spread of the disease to other areas.

The humoral immune response observed in such systems is necessarily a mixed response characterized by various antibodies directed against a number of different antigens

(6,9). Changes in antibody response to one particular antigen during the course of the infection may well be overshadowed by changes in antibody response against other antigens. Therefore, such systems are of little value for the follow-up of the development of the antibody response during the course of an infection (12).

Therefore, in this study crude antigen was used to design a sensitive and specific method. A different source was used to obtain the proper antigen derived from human and sheep as the best source (8).

There are various reports stating that the sensitivity of serological tests using crude cyst fluid as compared to the purified antigen is higher, since it is possible to lose the number of specific antigen in the purification process (2).

However, hydatid fluid collected from *E. granulosus* cysts is the most sensitive and probably the most widely antigen used for serological diagnosis of CE (3, 4).

In a study conducted using specific antigen (Arc5) in ELISA test, higher sensitivity and specificity (96.2% and

98%, respectively) were obtained. However, with the crude antigen these were lower (18.1% and 96%).

During the follow-up period, it is important that quantitative techniques with higher reproducibility and sensitivity be performed (7). In this study, IgG ELISA and CCIE were the most sensitive techniques for the pre-operative diagnosis of the hydatid disease (95.5% and 90%, respectively). Different sensitivities have been reported according to the methodology, however, ELISA provides quantitative results.

Therefore, in this study the high sensitivity and specificity could be attributed to lower concentrations of antibody and antigen used (1:100 dilution, 6µg/ml) and also probably due to the use of different substrates, i.e. TMB (3,3',5,5',Tetramethylbenzidine). In other studies the antigen concentration was higher (18 µg/ml) and the substrate used was OPD (1,2 Pheylene Diamine Hydrochloride). Arc5 antigen was used in three different assays (IHA, CIEP, IgG ELISA); the results revealed that IgG ELISA is superior to the other tests used (5).

In this study, the high specificity (96.6%) and sensitivity (93.3%) of IgG ELISA and CCIE indicated that CCIE

with sensitivity (90%) and specificity (100%) could be coupled as a confirmatory diagnostic procedure for hydatid disease.

Finally, the results of this study showed that the crude antigen used was more immunologic and had wide applications with good results. Other advantages of ELISA kit designed in this study were that it was easy to

Table 1. Age and sex distribution of patients, according to cyst location

Age (years)	Female			Male			Total
	Lung	Liver	Other	Lung	Liver	Other	
0-20	0	1	0	1	2	0	4
21-40	1	4	1	2	3	1	12
41-60	1	3	1	2	2	2	11
> 60	1	0	0	1	0	1	3
Total	3	8	2	6	7	4	30

perform, not expensive, and at the same time reliable results could be obtained with good sensitivity and specificity.

ACKNOWLEDGEMENTS

We are grateful to Mr. Vatankeh and Mrs. Hovhanessian for their technical assistance, advice and support.

Table 2. Comparitive diagnostic of ELISA and C.C.I.E for 30 patients with surgical proven hydatidosis

Sera	ELISA		C.C.I.E	
	Pos.	Neg.	Pos.	Neg.
	No.(%)	No.(%)	No.(%)	No. (%)
No. Positive (n = 30)	28(93.3)	2(6.7)	27(90)	3(10)
No. Negative (n = 30)	1(34)	29(96.6)	0	30(100)
No. other parasitic Infection (n = 30)	10(33.3)	20(66.7)	15(50)	15(50)

Table 3. Cross-Reaction

Groups	
No. of sera tested	No. of Positive reaction
Fasciolasis (n =15)	7
Toxocariasis (n=4)	1
Toxoplasmosis (n = 5)	1
Liver Cirrhosis (n= 6)	1
Total (n = 30)	10
Cross-Reaction (%)	33.3%

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