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Original Article

Evaluation of Specific IgG Antibody Detection in Diagnosis and Post Surgical Monitoring of Cystic Echinococcosis

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Abstract

Background: Cystic echinococcosis (CE) is one of the most predominant parasitic zoonosis infections in the world. Due to the high recurrence rate of the disease after surgery, follow up of the patient is necessary. The aim of current research was to evaluate the sensitivity of ELISA method for hydatid antibodies detection in confirmed CE cases before and its pattern after surgery.
Methods: The sensitivity of ELISA method was assessed for hydatid antibody detection in 143 pathologically confirmed CE cases. The existence of CE antibody in 69 confirmed CE cases were followed up for 6 to 12 months and up to 48 months in some cases.
Results: Sensitivity of ELISA was 90.2%. Fifty percent of seronegative cases had lung infection. All of the CE cases who were monitored for antibodies assessment after surgery showed variable levels of decrease in antibody titer. However, this decrease was not constant, regular and occurred at different times after surgery. Increasing antibody levels were occurred in few cases after surgery for up to 6 months.
Discussion: The present study showed that the sensitivity of ELISA for antibody detection against purified antigen B was high. Furthermore, this test is a useful and valuable index for post monitoring of CE patients after surgical treatment.

Keywords: *Cystic echinococcosis*, *Hydatid cyst,* Echinococcus granulosus, *ELISA.*

Introduction

Cystic echinococcosis is the larval stage of *Echinococcus granulosus*, which is one of the most predominant worldwide parasitic zoonosis infections. Diagnosis relies on combination of the imaging lesions by ultrasound or computed tomography and serological tests. Different serological tests for the diagnosis of CE have been developed and evaluated, but a wide variation of sensitivity and specificity has been reported. Results obtained vary according to the technique used, characteristics, number and location of hydatid cyst, different host, local parasite strain, biologic interaction and immune status of the patient. Among the various techniques employed, antibody detection is still a more reliable test for CE detection and follow up (1-4).

Although chemotherapy may be used in some cases of CE but, surgery is still the main treatment of hydatid cyst (5, 6). Local recurrence or secondary infection during surgery has been reported in more than 30% of cases. Likewise, during chemotherapy, it is difficult to ascertain progress of treatment. Therefore, monitoring of CE patients after surgery and during chemotherapy has been emphasized. Despite limitation of serological tests, due to their cost effectiveness and improvement facilities they are probably best choice for follow-up assessment of CE after either surgery and/or chemotherapy.

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In addition, due to exposure of CE in different organs, imaging methods have their limits of CE detection. Several authors have tried to establish serological follow-up assessment but these tests have their limited diagnostic values (7-11). Furthermore, due to variation in immune response among CE patients as a result of species variation of *E. granulosus* in different location in the world, assessment of serological antibody test in every endemic region should be considered separately.

The aims of this project were to assess the sensitivity of ELISA method in antibody detection among confirmed CE cases and it's evaluation for post surgical follow up.

Materials and Methods

Human serum samples

In a cross-sectional study one 143 serum samples were collected from confirmed CE patients from southwest of Iran before surgery. Antibody detection was performed among 69 CE patients every 6-12 months after surgery and followed up for up to 48 months in some cases. All sera were stored at -70 ^{0}C until used.

As control group, 300 serum samples were collected from healthy persons, in endemic area who were visited by specialist doctor. They were matched by gender and age with CE patients.

Antigen preparation

Purified antigen B was prepared from hydatid cyst fluid of sheep (12). Briefly, 200 ml of sheep hydatid cyst fluid were dialyzed against 0.005 M acetate buffer, pH 5.0, overnight and centrifuged at 50000 g for 30 min. The precipitate was then dissolved in 20 ml of 0.2 M phosphate buffer, pH 8.0. The preparation was then boiled in a water bath for 15 min and finally centrifuged at 50000 g for 60 min. The supernatant was designed as antigen B. Protein concentration of antigen B was determined by Bradford assay (13) from Bio-Rad Company. Optimal working concentration of antigen was assessed by checkerboard titration.

Assay procedure

Microplates (Immulon I, Dynatech) were coated with 5µg protein concentration of antigen B and plates were incubated overnight at 4 ^{0}C. Then plates were washed with PBS +0.1% Tween 20 three times and blocked for 1 h in PBS+5% skimmed milk. Plates were washed as above. Serum samples were added in 1:100 dilutions and incubated 1 hour at room temperature. Plates were washed as above and alkaline phosphates conjugated anti-human IgG (Sigma) was added (1:2000) and incubated 1h at room temperature. After washing as above Para-nitrophenyl phosphate (Sigma) was added and after 20 minutes, the plates were read by ELISA reader MRX (Dynatech) at 405 nm. ELISA specificity was 97%. CE sera were assigned antibody positive when their optical density were more than 0.350 (cut off point according to normal human sera).

Descriptive statistical analysis was used for all variables. Optical density (OD) cut off point was calculated as mean +2 standard deviation (SD) of normal human sera.

Results

From 143 CE sera evaluated by ELISA for hydatid antibody detection against purified antigen B, 128 (90.2%) were positive. Fifty percent of seronegative patients had lung infection. (Table 1)

The most predominant organs involved were liver and lung with incidence of 65.3% and 36.36%, respectively. Distribution of CE among different age groups showed the predominant age infection in 21-40 yr (39.79%) and in about more than 40 (33.66%) yr age groups (Table 1).

CE infection was detected in 62.9% and 37.1% in woman and man, respectively. Decreasing antibody levels did not occurred at the same rate in all patients. For example in two patients decreasing level was observed after three months while it occurred after 48 months in one patient with lung infection.

However antibody reduction was detected in all CE patients after surgery but during different

duration. Thirteen (18.84%) patients become negative for antibody levels against purified antigen B (Table 2).

Thirty six cases had history of previous CE. These patients had recurrence of CE in liver (20 cases), lung (10 cases) and liver and lung (6 cases). Eighty (55.94%) of CE cases had contact with dog, either keeping dog at home or in their environment. In addition, 50 (34.96%) cases used to slaughter at home.

Table 1: Distribution of hydatid cysts in various organs and results of antibody detection against purified antigen B in confirmed CE patients

Cyst location / Antibody detection	Liver	Lung	Liver & lung	Spleen	Liver & Spleen	Other organs	Total
Positive	77	35	11	1	1	4	129
Negative	5	7	-	2	-	-	14
Total	82	42	11	3	1	4	143

Table 2: The time lapse for developments seronegativity among 13 patients with confirmed CE after surgery

Duration (months) on IgG negativity	48	22	16	15	13	11	3
Number of patients	1	1	1	1	5	2	2

Discussion

There is no safe and direct sampling collection method for detection of hydatid infection and acceptable immune response induced in intermediate hosts. Therefore, imaging methods and serological tests has been used widely for CE (14-19). Due to the non-specific nature of the clinical symptoms and the limitations of imaging methods, a reliable standard serological test is invariably necessary. The present study indicated a high sensitivity (90.2%) for CE diagnosis using antibody detection against purified antigen B by ELISA. Different sensitivity rates have been reported previously (8, 9, 17, 19-22), but in agreement with our results, antibody detection against antigen B in ELISA is the most sensitive and specific serologic test for CE (6, 7, 17, 23). In the present study 9.8% CE cases were seronegative. Seronegativity of confirmed CE cases has been reported up to 40% (24-27). In addition, our results showed that 50% of seronegative cases had lung infection. This finding supports the low antibody response in lung infection (15-19, 27-29). However no clear explanation for seronegativity in CE cases has been offered, but lack of antibody response in some of CE cases may be due to possible strain variation of *E. granulosus*, host immune response, cyst location, number and cyst situation, role of immune complexes and some other factors (17). Predominant involvement of CE in liver and lung is similar to other reported investigations. High rate (67.42%) of CE in age groups above 20 years old in the present study may be due to greater chance of exposure of adults to contamination and/or prolonged of incubation period of infection. There was increasing antibody levels after surgery in few cases, presumably because of antigen release. Increasing antibody concentration after CE treatment in individual CE cases has been reported in previous studies (7, 10, 11, 29).

But decreasing antibody levels occurred in all these cases after a longer period (up to 6 months). Our results indicated decreasing antibody levels against purified antigen B in all CE cases after surgery and even lack of antibody in 13 (18.84%) cases. But this reduction did not show constant and similar patterns in all CE cases. Our data agree with some other studies (15, 29), but there was a wide variation of duration of antibody reduction that occurred from 3 to 48 months. Decreasing antibody levels in CE patients after chemotherapy treatment or surgery has been reported during a wide duration range from 2 months up to 11 years (5, 9, 10, 16, 29). There are different hypothesis for fluctuation of antibody levels after treatment in CE patients such as sensitivity of applied serologic tests, more sensitive tests may become negative after longer period, possibility of CE recurrence in patients especially after surgery, releasing of antigenic materials during treatment or after surgery.

In conclusion, despite the variability in duration of reduction of antibodies after surgery among CE patients, the present study confirmed the high sensitivity of ELISA using purified antigen B for serologic CE cases and support its valuable role for post monitoring of CE after surgery. Therefore due to limitation of imaging methods ELISA may be one the best choices for post monitoring of CE, especially in endemic areas.

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