Diagnostic sensitivity of enzyme-linked immunosorbent assay for IgG in hydatid disease

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Abstract

Context Hydatid disease in humans is most commonly caused by *Echinococcus granulosus* and results in development of cysts in various organs of body. The diagnosis is made by serology i.e. by estimation of antibody levels or on imaging studies. Enzyme-linked immunosorbent assay (ELISA) is widely used for serological confirmation of the disease.

Aim To study the sensitivity of ELISA for IgG antibodies against *E. granulosus* in detecting hydatid disease of liver or lung.

Settings A retrospective study of serology of all surgically confirmed cases of hydatid cysts of liver or lung in a tertiary level hospital.

Methods and material The levels of IgG antibodies against *Echinococcus*, measured by ELISA, in patients of cystic lesions of liver or lung, who underwent surgery for the same and confirmed as having hydatid disease, during surgery or on histopathological examination after surgery, were recorded and analysed. There were 28 such patients from January 2001 to 2007. Twenty-three patients with hy-

datid cysts in liver and 4 in lung were included. One patient with hydatid liver was excluded from the study due to heavily calcified cyst.

Results Two types of kits were used; one using crude antigen and other using purified antigens. Among 23 patients with hydatid cysts of liver, 8 had positive serology while 15 had either equivocal or negative results. All 4 patients with hydatid of lung had positive serology.

Conclusion ELISA test is not sensitive enough to be relied upon for confirmation of hydatid disease and considering its high cost, an alternative more specific, sensitive, cheaper and easily available test needs to be evaluated for confirming hydatid disease.

Keywords Hydatid disease · ELISA

Introduction

Humans are potential intermediate hosts of hydatidosis; an infection caused by cestodes of the genus *Echinococcus* and get infected by ingesting eggs passed by the infected animal in their faeces. The larva of the cestodes gets into portal system through intestines and lodge in various organs of the body. Liver is most common site followed by lungs, brain, muscle or any other body parts [1]. Presentation to surgery is for alleviation of symptoms caused by pressure effects of the cyst and hence a definitive diagnosis is mandatory to prevent risks of rupture of the cyst.

Clinical signs and symptoms guide the investigations. Since *Echinococcus* eggs are not shed by infected humans, serological determination is used for confirmation of the disease. Ultrasound scanning is widely available in India

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but it is only indicative of the disease and not diagnostic. CT scan and MRI may clinch diagnosis but are either too costly or not readily available hence serological testing is usually relied upon for confirmed diagnosis of hydatid disease.

The serological tests in use include enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence antibody test, immunoelectrophoresis and immunoblotting. Among these ELISA is most widely available.

ELISA for *Echinococcus* IgG is the quantitative determination of IgG antibodies against *E. granulosus* and *E. multilocularis* in human serum and though tests measuring different subtypes of the IgG antibodies are mentioned, the ones commercially available measure the total IgG antibodies against *Echinococcus* in the patients. For detection of antibodies against *E. granulosus* infection, crude or purified antigens are used and by purification of the antigens, specificity of the test increases significantly. The reported sensitivity of this test varies from 88.23–100% but doubts remain [2].

The objective of the study was to assess the results of ELISA for IgG for hydatid of patients of surgically or histopathologically proven hydatid cysts of liver or lung and to calculate the actual sensitivity of the test.

Methods and materials

In this retrospective study, patients who underwent surgery for suspected hydatid cysts of liver or lung from January 2001 to January 2007 in a tertiary level hospital in North India were evaluated. All these patients had undergone preoperative ELISA for IgG levels for *Echinococcus*.

There were 28 patients in whom a diagnosis of hydatid disease was confirmed either by intraoperative findings or by postoperative histopathology. The data of all these patients was recorded irrespective of their age, sex, clinical presentation or preoperative imaging.

The inclusion criteria were a preoperative estimation of IgG for hydatid disease and confirmation of hydatid disease during surgery or on postoperative histopathological examination. One patient among these 28 was excluded as his contrast enhanced CT scan done before surgery showed a heavily calcified cyst in the liver, suggestive of a dead cyst.

The IgG levels had been estimated by two types of standard commercially available kits.

- 1) ELISA *Echinococcus* Microwell Serum ELISA Diagnostic Automation (23961 Craftsman Rd, Suite E, Calabasas, CA 91302). The micro test wells are coated with a crude antigen from an *Echinococcus* cyst.
- 2) Ridascreen *Echinococcus* IgG (R-Biopharm AG, Landwehrstr, 54, D-64293 Darmstadt, Germany). The micro test wells are coated with purified antigens.

The Microwell kit was been used on 20 patients and the Ridascreen kit was used on 7 patients.

Results

The study was conducted on data over a period of 6 years. There were 8 males (6-hydatid of liver and 2-hydatid of lungs) and 19 females (17-hydatid of liver, 2-hydatid of lung) in the study group (Table 1).

The median age was 35 years. Twenty-three patients had cystic lesions in liver (15-right lobe, 5-left lobe, 3-both lobes). 4 patients had cystic lesions in the lungs (2-left lung, 2-right lung).

Out of 23 patients with hydatid cyst of liver, 7 underwent USG abdomen with reports suggestive of cystic lesions in liver with (2 patients) or without (5 patients) multiple septa. Fifteen patients of liver cysts underwent CT scan i.e. either plain (4 patients) or contrast enhanced (11 patients) and only in four reports, the diagnosis was hydatid disease. One patient underwent MRI of abdomen and was reported as having hydatid cyst of liver.

The 4 patients of hydatid lung underwent CT scan of the chest i.e. either plain (1 patient) or contrast enhanced (3 patients) and all were reported as having single cyst in lung with no septa and a doubtful clinical diagnosis.

Two types of kits were used for estimation for IgG levels in these patients. The Microwell kit was used in 20 patients and Ridascreen kit on 7 patients.

Out of 23 patients of hydatid cysts of liver, 8 had a positive serology another 8 had equivocal serology and 7 had negative serology (Table 2). The Microwell kit had been used on 17 patients with positivity rate of 23.5% while Ridascreen kit was used on 6 patients with positivity rate of 66.6%. The combined sensitivity of the test by either of the two kits was 37.4%.

All 4 patients of hydatid lung had positive serology for IgG (Table 3). Microwell kit was used on 3 patients while

Table 1 Showing sex distribution in the study group

	Males	Females
Hydatid cyst of liver (n = 23)	6	17
Hydatid cyst of lung (n = 4)	2	2
Total (n = 27)	8	19

Table 2 Serology tests in patients of hydatid cysts of liver

	Positive	Equivocal	Negative	Sensitivity (%age)
Microwell (n = 17)	4	7	6	23.5%
Ridascreen (n = 6)	4	1	1	66.1%
Total (n = 23)	8	8	7	34.7%

Ridascreen kit had been used in 1 patient. Thus the sensitivity of the test in these patients was 100%.

The overall sensitivity of ELISA in both groups of patients tested with either of the two kits was 44.4% (Table 4).

Discussion

There are four species of genus *Echinococcus* which are of clinical importance. *E. granulosus* (causes cystic disease), *E. multilocularis* (causes alveolar disease) and *E. vogeli* and *E. oligarthus* (causes polycystic disease). Humans are accidental intermediate hosts and population at high risk include the ones where dogs are used to herd sheep and are intimate members of the family [2].

In human beings, the eggs hatch in duodenum, after exposure to gastric and intestinal enzymes and the released oncospheres penetrate the intestine and are carried by the blood stream to be filtered into various parts of body [2]. Sixty percent of such cysts are found in the liver followed by lung, brain, muscles etc. [1].

The majority of these cysts produce symptoms depending on the site of localisation. Patients present in out-patient or emergency as a consequence of pressure effects produced by the expanding cysts which grow at an average rate of 1 cm per month [3].

Serology and imaging are the primary modes of diagnosis. Some authors have used fine-needle aspiration (FNAC) of the cyst for confirmation of the disease with no reported complications but further studies are required before FNAC can be used on large scale [4].

The definitive diagnosis of cystic *Echinococcus* is by combination of CT or MRI with serological testing. USG is indicative of the disease but not diagnostic. But CT and MRI may not be available or be too costly and additionally, as

shown by this study, most reports of such imaging are inconclusive.

In serology, the detection of circulating *E. granulosus* antigens in sera is less sensitive than antibody detection, which remains the method of choice [5].

Insensitive and non-specific tests like Casoni intradermal test, complement fixation test, indirect haemagglutination test and latex agglutination test have been replaced by ELISA, indirect immunofluorescence antibody test, immunoelectrophoresis, and immunoblotting [6].

Among the above, ELISA for detection of IgG antibodies is the most commonly used. It is considered to be highly sensitive and specific in detecting anti-*Echinococcus* antibodies irrespective of the site of cyst localisation [7].

False negative ELISA has been reported in small cysts (e.g. early after infection), intact cysts (i.e. no leakage of the immunogenic cyst fluid), cysts in extra hepatic locations and heavily calcified cysts (e.g. non-viable) [8].

Kaur et al. compared the specificity and sensitivity of standard ELISA, rapid ELISA and the indirect haemagglutination test in patients of hydatid disease of liver [9]. They reported the specificity of 100%, 88.23% and 100% respectively. No cross reactions were observed with rapid ELISA technique using samples from cysticercosis and amoebiasis patients and normal healthy controls. The reported sensitivity of standard ELISA was 88.23%.

According to Wattal et al, among purified antigens, ELISA for IgG was the most sensitive (96.5%) and ELISA for IgE was least specific (24.1%) [7].

Rigano et al. reports that antibody measurement is also valuable in monitoring a patient of hydatid disease after treatment for any recurrence or relapse. In patients undergoing successful removal of hydatid cyst, the IgG4 subclass becomes negative soon after surgery and those with relapsing disease maintain high IgG4 titres on ELSIA. This suggests that IgG4 subclass is a good marker for hydatidosis follow-up [10].

But results of our study are in contrast to the reported figures in various studies.

In case of hydatid cyst of liver, in contrast to the reported sensitivity of 88.23% [8] to 100% [7], our results showed a sensitivity of only 34.7% while the rest had either equivocal or negative serology.

Pulmonary hydatosis is associated with poor seropositivity with some reports suggesting 48.5% positivity rate [11], attributed to production of low levels of antibodies in these patients. But in our study all the 4 patients had positive serology.

The reported high sensitivity of tests using purified antigens was reproduced in this study as well (Table 4). Patients subjected to ELISA testing using Microwell kit, which uses crude antigens, showed sensitivity of 35%, while patients subjected to ELISA testing using Ridascreen kit, which uses purified antigens, had sensitivity of 71.4% but the later was not being widely used and this could be one reason for the overall decreased sensitivity of ELISA as being shown in this study.

Table 3 Serology tests in patients of hydatid cysts of lung

	Positive	Equivocal	Negative	Sensitivity (%age)
Microwell (n=3)	3	0	0	100%
Ridascreen (n=1)	1	0	0	100%
Total (n=4)	4	0	0	100%

Table 4 Combined results of serology comparing the two kits

	Positive	Equivocal	Negative	Sensitivity (%age)
Microwell (n=20)	7	7	6	35%
Ridascreen (n=7)	5	1	1	71.4%
Total (n=27)	12	8	7	44.4%

The kits used for the tests were of international quality and hence the high numbers of false negatives due to poor quality enzyme conjugated anti-immunoglobulin, as reported by some reports is unlikely [7].

Conclusion

ELISA test for IgG is the most commonly employed test for diagnosis of hydatid disease but the reported high sensitivity of the test could not be reproduced in our study. Kits using purified antigens showed higher sensitivity but they were not being as widely used as the ones using crude antigens. Sole dependence on such inconsistent test for diagnosis of the hydatid disease should be avoided and wherein indicated, kits using purified antigens only should be used.

Considering its high cost i.e. average Rs. 600/-, a newer, more sensitive and cheaper, serological or nonserological test needs to be evaluated for confirmation of the disease.

Conflict of interest The authors do not have any disclosable interest

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