Tehran University of Medical Sciences Publication
http:// tums.ac.ir

Iranian J Parasitol

Open access Journal at
http:// ijpa.tums.ac.ir

Iranian Society of Parasitology
http:// isp.tums.ac.ir

Original Article

Performance of Antigen B Isolated from Different Hosts and Cyst Locations in Diagnosis of Cystic Echinococcosis

***H Rahimi*[1], *SM Sadjjadi*[1], *[*]*B Sarkari*[2]**

[1]*Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
[2] *Center of Basic Researches in Infectious Diseases, Shiraz University of Medical Sciences, Shiraz, Iran*

(Received 25 Oct 2010; accepted 18 Jan 2011)

ABSTRACT

Background: The aim of this study was to assess the performance of Antigen B (AgB) isolated from different *Echinococcus granulosus* intermediate hosts and from different cyst locations for immunodiagnosis of human cystic echinococcosis (CE).
Methods: Hydatid cyst fluids were collected from lung and liver cysts of sheep, liver cysts of goats, lung cysts of camels and cattle, and liver cysts of human. AgB was purified from each of these hydatid cysts fluids. Serum samples obtained from 47 pathologically confirmed cases of CE along with 30 sera samples from non-CE patients and 40 sera from healthy individuals were tested by ELISA using AgB prepared from different hosts or cyst locations.
Results: The highest sensitivity (97.8%) for diagnosis of CE was seen with AgB prepared from human liver cysts. This maximal sensitivity was followed by AgB isolated from those of sheep liver and lung cysts. The least sensitivity was found with AgB prepared from bovine lung cysts. The highest specificities (97.1%) were observed with AgB isolated from human liver cysts followed by those of sheep and goat liver cysts while the lowest specificity was seen with AgB isolated from bovine lung cysts. In view of the specificities and sensitivities of the different AgB, the best validity was found for AgB prepared from human liver cysts while the least validity was found with AgB prepared from bovine lung cysts.
Conclusion: For any AgB-based tests, obtaining of the antigen from one of these sources will significantly increase the diagnostic sensitivity and specificity of the assay.

Keywords: *Hydatidosis, ELISA, Serodiagnosis, Antigen*

[*]**Corresponding author: Tel:** # 98- 7112305291, **Fax:** #98-7112305291, **E-mail:** sarkarib@sums.ac.ir

Introduction

Cystic echinococcosis (CE) is a zoonotic infection of humans and domestic animals caused by the larval stage of the dog tapeworm *Echinococcus granulosus*. Intermediate hosts (such as goats, sheep, and camels) are infected by ingesting eggs, which passed through the feces of the definitive hosts, usually feral dogs (1). Humans become infected as accidental hosts through egg ingestion as well.

Despite the development of sensitive and specific methods, the immunodiagnosis of CE remains a difficult task (2-3). A majority of available screening tests give a high percentage of false negative results (up to 25%) while false positive reactions due to infection with other cestoda or even other helminthes can be seen in different assays which have been used for diagnosis of CE (4-5).

Different antigens in different serological assays have been used for immunodiagnosis of human hydatidosis (4). Using hydatid cyst fluid (HCF) as an antigen reduces the specificity of the assay since HCF contains various metabolites of the host and the parasite. Antigen B (AgB) is one of the main antigens of HCF, which has been extensively used in immunodiagnosis of CE (5-9). AgB is a thermo-stable lipoprotein that dissociate in SDS-PAGE into three bands of 8-12, 16, and 24 kDa (6).

It has been demonstrated that AgB is encoded by at least five main gene clusters, *EgAgB1-5,* and the isoforms of related proteins vary 44–81% in amino acid sequence (6). One of the reasons that different laboratories get different results by using the AgB for diagnosis of CE might be the differences in nature and quality of prepared antigen (6).

Considering the differences in AgB from different hosts or cyst locations, the performance of a given diagnostic assay, which uses this antigen, might also be different. The current study was designed to assess the performance of AgB isolated from different *E. granulosus* intermediate hosts and from cysts in different locations of the hosts for immunodiagnosis of human CE.

Material and Methods

Preparation of Hydatid Cyst Fluids

Hydatid cyst fluids (HCF) were collected from lung and liver cysts of sheep, liver cyst of goat, lung cysts of camels and cattle, and liver cyst of human (from Al-Zahra Hospital, Isfahan). Camel hydatid cysts were obtained from Najafabad, Isfahan abattoir while bovine and ovine cysts were obtained from Shiraz abattoir. To remove the protoscoleces and large particles, HCF was centrifuged at 1000 g for 30 min. The cattle hydatid cysts were unfertile while the other cysts were fertile. Hydatid cysts from different hosts or locations were kept separately and used for preparation of antigen B. Protein content of the sample was determined and the collected antigen was stored at -20 °C until use.

Preparation of Antigen B

Antigen B was prepared from HCF, collected from different hosts and different cyst locations (5). Briefly, 100 ml of HCF were dialyzed overnight against 5 mM acetate buffer (pH 5) at 4 ºC. The samples were centrifuged at 50,000 g for 30 min to remove the albumin. The supernatant was discarded and the pellet was dissolved in 0.2 M phosphate buffer (pH 8). Saturated ammonium sulfate was used to take out the immunoglobulin from the sample. Finally, the sample was boiled in a water bath for 15

min and centrifuged at 50,000 g for 60 min to isolate AgB, which is heat stable, from other antigens. The quality of antigen B and presence of AgB subunits was checked by SDS page and Coomassie staining.

Serum Samples

Forty-seven serum samples were obtained from surgically and pathologically confirmed CE patients from Al-Zahra Hospital at Isfahan University of Medical Sciences. Twenty-seven of cases had hydatid cyst in their liver and 20 cases had lung cysts. The samples were collected from patients before, during, or shortly after operation. Moreover, 30 sera samples were collected from patients with ascariasis (10 samples), strongyloidiasis (3 samples), trichostrongilosis (7 samples) and patients with malignant diseases (10 samples). As negative control, 40 sera samples from healthy subjects were also collected. Ethical approval of the study was given by the Ethics Committee of Shiraz University of Medical Sciences and consent was obtained from the participants.

Enzyme linked Immunosorbent Assay (ELISA)

To find out the performance of AgB, which were isolated from different hosts and different cyst locations, the purified antigens were evaluated in an ELISA system. ELISA was carried out in flat-bottom 96-well microplates (Nunc, Nalgene, Nunc International, Roskilde, Denmark). The plates were coated with 5 µg/ml of each purified AgB (100 µl/well) in coating buffer (0.05 M carbonate-bicarbonate buffer, pH 9.6) and incubated at 4 ºC overnight. Excess antigen was removed by washing the plate five times in phosphate buffered saline-Tween 20 (PBST, pH 7.4 containing 0.05% Tween 20). Blocking was made with 3% skimmed milk in PBST for 2 hours. The wells were washed as before and 100 µl of serum samples (1/100 dilution in PBST) from surgically confirmed cystic echinococcosis patients along with samples from healthy subjects as negative controls and sera from ascariasis, strongyloidiasis, trichostrongilosis and malignant patients were applied to the plates and incubated for 1.5 hour at room temperature. The plates were washed as before and 100 µl of horseradish peroxidase conjugated antibody against human immunoglobulin (Dako; Denmark A/S, Glostrup, Denmark) at 1/3000 dilution in PBST were added to the plates and incubated for 1 hour at room temperature. After washing as before, the plates were incubated with chromogen/substrate (100 µl/well of OPD, 0.025% H_2O_2 in 0.1 M citrate buffer, pH 5) and the reaction terminated with 1mM sulphuric acid after 30 min. The absorbance at 490 nm was checked with an ELISA microplate reader. For each antigen, the cutoff value, which differentiates positive from negative results, was set by defining the cutoff as the mean value of the normal serum group plus three standard deviations.

Results

The quality of antigen B and presence of AgB subunits was checked by SDS page and Coomassie staining. Fig. 1 shows the profile of antigen B isolated from different hosts. The concentration of AgB, using the same initial volume of HCF, for each hosts or cyst locations were as follow: sheep liver 650, goat liver 550, human liver 280, camel lung 76, cattle lung 70 and sheep lung 390 µg/ml. Using AgB prepared from sheep liver cysts, 45 (95.7%) of hydatidosis patients were found to be positive while 3 sera from negative controls and two from non-CE patients were also found to be positive. Therefore, the sensitivity and specificity of this antigen for diagnosis of CE were 95.7% and 92.8% respectively. When AgB prepared from sheep lung cysts was used in ELISA,

sensitivity and specificity of this antigen for diagnosis of CE were 93.6% and 88.5% respectively.

Using AgB purified from goat liver cyst, antibodies to this antigen were detectable in 43 (91.4%) of sera of CE patients and in 2 (5%) of negative control sera. Accordingly, a sensitivity of 91.4% and specificity of 92.8% was determined for this antigen. Using AgB prepared from human liver cysts, 46 (97.8%) of CE patients were found to be positive. No cross reactivity with control sera were observed with this antigen but sera from two of non-CE patients reacted with this antigen. The sensitivity and specificity of this antigen were 97.8% and 97.1% respectively. With AgB from camel lung cysts, 44 (93.6%) of CE patients were found to be positive and three (7.5%) of negative controls were false positive.

Using AgB prepared from bovine lung cysts, antibodies were detected in sera of 37 (78.5%) CE patients while five (12.5%) of negative controls were also reacted with the antigen. Sensitivity and specificity of 78.7% and 85.7% was obtained for this antigen. When a cocktail of AgB prepared from six CE intermediate hosts or cyst locations was used, a sensitivity of 72.2% (95% CI: 56.9-84.5) and specificity of 96.6% (95% CI: 87.4-99.4) were seen.

The performances of different AgB in diagnosis of hydatid cyst in this study are shown in Table 1. The highest sensitivity for diagnosis of CE was seen with AgB prepared from human liver cysts. This maximal sensitivity was followed by AgB isolated from those of sheep liver and lung cysts. The least sensitivity was found with AgB prepared from bovine lung cyst. The highest specificities were observed with AgB isolated from human liver cysts followed by those of sheep and goat liver cysts while the lowest specificity was seen with AgB isolated from bovine lung cyst. Diagnostic sensitivities and specificities of each AgB in diagnosis of CE are listed in Table 2. In view of the specificities and sensitivities of the different AgB, the best validity was found for AgB prepared from human liver cysts while the least validity was found with AgB prepared from bovine lung cysts. Considering the cyst location in human, AgB prepared from sheep liver cysts and human liver cysts have been able to diagnose both liver and lung cysts in CE patients. Table 3 shows the details of AgB-based detection of human liver and lung cysts.

Statistical analysis

While a substantial agreement (Kappa= 0.78) was found between ELISA method using sheep liver and human liver antigens, the lowest agreement (Kappa= 0.14) was found between AgB from bovine lung and human liver cysts.

Table 1: Serum samples tested by ELISA, using different AgB, for immunodiagnosis of human cystic echinococcosis

Source of antigen	CE patients			Negative control samples			Non-CE patients		
B	No.	Positive	Percent	No.	Positive	Percent	No.	Positive	Percent
Sheep liver cyst	47	45	95.7	40	3	7.5	30	2	6.6
Goat liver cyst	47	43	91.4	40	2	5	30	3	9.9
Human liver cyst	47	46	97.8	40	0	0	30	2	6.6
Camel lung cyst	47	44	93.6	40	3	7.5	30	4	13.2
Bovine lung cyst	47	37	78.5	40	5	12.5	30	5	16.5
Sheep lung cyst	47	44	93.6	40	3	7.5	30	5	16.5

Table 2: Diagnostic sensitivities and specificities of different AgB in diagnosis of human CE

Source of antigen B	Sensitivity (percent)	Specificity (percent)
Sheep liver cyst	95.7 (95% CI:84.2-99.2)	92.8 (95% CI:83.4-97.3)
Goat liver cyst	91.4 (95% CI:78.2-97.2)	92.8 (95% CI:83.4-97.3)
Human liver cyst	97.8 (95% CI:87.2-99.8)	97.1 (95% CI:89.1-99.5)
Camel lung cyst	93.6 (95% CI:81.4-98.3)	90 (95% CI:79.8-95.5)
Bovine lung cyst	78.7 (95% CI:63.9-88.8)	85.7 (95% CI:74.8-92.5)
Sheep lung cyst	93.6 (95% CI:81.4-98.3)	88.5 (95% CI:78.1-94.5)

Table 3: Diagnosis of human hydatid cyst in lung and liver by AgB isolated from different hosts

Source of antigen B	CE patients with liver cyst (No.=27)		CE patients with lung cyst (No.=20)	
	Positive No. (%)	Negative No. (%)	Positive No. (%)	Negative No. (%)
Goat liver cyst	25 (92.6)	2 (7.4)	18 (90)	2 (10)
Sheep liver cyst	26 (96.3)	1 (3.7)	19 (95)	1 (5)
Human liver cyst	27 (100)	0 (0)	19 (95)	1 (5)
Camel lung cyst	26 (96.3)	1 (3.7)	18 (90)	2 (10)
Bovine lung cyst	22 (81.5)	5 (18.5)	16 (80)	4 (20)
Sheep lung cyst	26 (96.3)	1 (3.7)	18 (90)	2 (10)

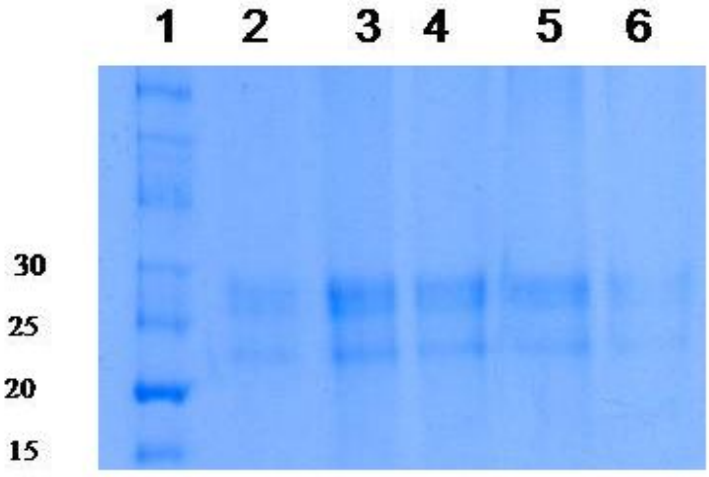

Fig. 1: Profile of antigen B (under reducing condition) isolated from different hosts. Line 1: Molecular marker, line 2 and 3 antigen B from sheep liver, line 4: goat liver, line 5: human liver, line 6: sheep lung

Discussion

Diagnosis of hydatid cyst is still problematic and the search for highly sensitive and specific antigens is the greatest challenges to overcome the unsatisfactory performance of the currently available immunological assays (4, 10).

One of the main antigens, which have been used extensively for diagnosis of CE, is HCF. For clinical practices, crude HCF has a high sensitivity, ranging typically from 75% to 95% (4). Nevertheless, HCF specificity is far from satisfaction since cross-reactivity with antigens of nematodes and trematodes is common (4, 10-14).

AgB, one of the main components of HCF, has been extensively used for diagnosis of CE. Recently we have used this antigen in comparison with HCF in counter current immunoelectrophoresis (CCIEP), ELISA and western blotting methods and have found a satisfactory performance for this antigen in diagnosis of CE in Iran (5, 9-10). Native and recombinant forms of this antigen have been evaluated in different studies for diagnosis of CE (15-16).

It is well known that the nature and quality of AgB in hydatid cyst fluid are variable among the host species. Antigen B family has putative isoforms that differ 44-88% in amino-acid sequences. Different isoforms may have different diagnostic features (6). This differences support the idea that any CE-endemic area might try to prepare and use the native antigen for routine diagnosis of CE (9).

In this study, reactivity of CE patient sera with AgB isolated from different hosts origin (human, sheep, goat, camel and cattle) or different cyst location (liver or lung) were compared simultaneously under the same condition in an ELISA system.

The best performance was seen with AgB isolated from human liver cysts followed by antigen from sheep liver cysts. Hydatid cyst fluid from human cysts contains host proteins including immunoglobulin but these proteins will be removed from the fluid during the procedures of isolation of AgB. It has been reported that AgB in human exploits the activation of T helper cells by eliciting a non-protective Th2 cell response. This in turn resulted in elevated specific immunoglobulin E (IgE) and IgG4 antibodies binding to AgB (17). Therefore it can be postulated that the antibodies which has been raised against AgB in human reacted well with this antigen. However obtaining hydatid cyst fluid from human cysts is not an easy task and therefore this source of AgB might not always be available for preparation of the antigen. The maximal performance of the AgB, after human liver cysts was belongs to sheep liver cyst.

The remarkable performance of AgB isolated from human liver cyst in diagnosis of CE might be due to the homogeneity of this antigen in human population as this recently pointed out by Tawfeek (18). They evaluated the genetic variability of AgB among *E. granulosus* in Egyptian isolates and reported a similar pattern of PCR-RFLP after restriction enzyme digestion in each host group (human, pig, camel and sheep). Their results revealed a high intra-group homogeneity of AgB.

The prominent strain of *Echinococcus granulosus* in human in Iran is G1 strain (19-20), which is a sheep-dog strain. Human CE patients raise antibodies against G1 strain, therefore AgB from sheep liver cysts must be an appropriate candidate for immunodiagnosis of CE in human, and our results clearly demonstrated this concept.

The lowest performance of AgB was seen with those isolated from bovine lung cysts. In Iran, only 19% of bovine cysts are fertile

(20). The lower rate of protoscoleces in bovine hydatid cysts might be accounted for weak performance of AgB isolated from these intermediate hosts for diagnosis of human CE.

For any given serological assay based on AgB, method of antigen preparation, differences in strain of parasite, location of the cyst, clinical status of the host and type of the cysts might be accountable for differences in results of the assays (6). In our study, AgB isolated from liver had a better performance in diagnosis of CE patients in comparison with AgB from other sources.

Taken together, although in this study all AgB isolated from different hosts and cyst locations generally exhibited relatively high diagnostic performance, however performance of AgB isolated from human liver cysts followed by sheep liver cyst were greater than the rest. Therefore, it can be suggested that for any AgB-based assays, preparing of the antigen from one of these sources will significantly increases the diagnostic sensitivity and specificity of the assay.

Acknowledgments

This investigation received financial support from the office of vice-chancellor for research, Shiraz University of Medical Sciences (Grant No. 4106). The authors declare that there is no conflict of interests.

References

1. Eckert J, Deplazes P. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. Clin Microbiol Rev. 2004; 17(1):107-135.
2. Ortona E, Rigano R, Buttari B, Delunardo F, Ioppolo S, Margutti P et al. An update on immunodiagnosis of cystic echinococcosis. Acta Trop. 2003; 85(2):165-171.
3. Siracusano A, Bruschi F. Cystic echinococcosis: progress and limits in epidemiology and immunodiagnosis. Parasitologia 2006; 48(1-2):65-66.
4. Carmena D, Benito A, Eraso E. Antigens for the immunodiagnosis of *Echinococcus granulosus* infection: An update. Acta Trop. 2006; 98(1):74-86.
5. Sadjjadi SM, Abidi H, Sarkari B, Izadpanah A, Kazemian S. Evaluation of enzyme linked immunosorbent assay, utilizing native antigen B for serodiagnosis of human hydatidosis. Iran J Immunol. 2007; 4(3):167-172.
6. Mamuti W, Sako Y, Nakao M, Xiao N, Nakaya K, Ishikawa Y et al. Recent advances in characterization of *Echinococcus* antigen B. Parasitol Int. 2006; 55:S57-S62.
7. Leggatt GR, Yang W, McManus DP. Serological evaluation of the 12 kDa subunit of antigen B in *Echinococcus granulosus* cyst fluid by immunoblot analysis. Trans R Soc Trop Med Hyg. 1992; 86(2):189-192.
8. de la Rue ML, Yamano K, Almeida CE, Iesbich MP, Fernandes CD, Goto A et al. Serological reactivity of patients with *Echinococcus* infections (*E. granulosus*, *E. vogeli*, and *E. multilocularis*) against three antigen B subunits. Parasitol Res. 2010; 106(3):741-745.
9. Sarkari B, Sadjjadi SM, Abidi H, Izadpanah A, Kazemian S, Rafati A. Application of western blotting using native antigen B for serodiagnosis of human cystic echinococcosis. Iran J Parasitol. 2007; 2(3):7-12.
10. Siracusano A, Buttari B, Delunardo F, Profumo E, Margutti P, Ortona E et al. Critical points in the immunodiagnosis of cystic echinococcosis in humans. Parasitologia 2004; 46(4):401-403.

11. Sadjjadi SM, Sedaghat F, Hosseini SV, Sarkari B. Serum antigen and antibody detection in echinococcosis: application in serodiagnosis of human hydatidosis. Korean J Parasitol. 2009; 47(2):153-157.
12. Ito A, Nakao M, Sako Y. Echinococcosis: serological detection of patients and molecular identification of parasites. Future Microbiol. 2007; 2:439-449.
13. van Doorn HR, Wentink-Bonnema E, Rentenaar RJ, van GT. Specific cross-reactivity in sera from cystic echinococcosis patients in an enzyme-linked immunoelectrotransfer blot for cysticercosis diagnostics. Trans R Soc Trop Med Hyg. 2007; 101(9):948-950.
14. Lorenzo C, Ferreira HB, Monteiro KM, Rosenzvit M, Kamenetzky L, Garcia HH et al. Comparative analysis of the diagnostic performance of six major *Echinococcus granulosus* antigens assessed in a double-blind, randomized multicenter study. J Clin Microbiol. 2005; 43(6):2764-2770.
15. Poretti D, Felleisen E, Grimm F, Pfister M, Teuscher F, Zuercher C et al. Differential immunodiagnosis between cystic hydatid disease and other cross-reactive pathologies. Am J Trop Med Hyg. 1999; 60(2):193-198.
16. McVie A, Ersfeld K, Rogan MT, Craig PS. Expression and immunological characterisation of *Echinococcus granulosus* recombinant antigen B for IgG4 subclass detection in human cystic echinococcosis. Acta Trop. 1997; 67(1-2):19-35.
17. Rigano R, Profumo E, Bruschi F, Carulli G, Azzara A, Ioppolo S et al. Modulation of human immune response by *Echinococcus granulosus* antigen B and its possible role in evading host defenses. Infect Immun. 2001; 69(1):288-296.
18. Tawfeek GM, Elwakil HS, Awad NS, El-Hoseiny L, Thabet HS, Sarhan RM et al. Genetic variability of antigen B among *Echinococcus granulosus* Egyptian isolates. Korean J Parasitol. 2009; 47(3):259-264.
19. Harandi MF, Hobbs RP, Adams PJ, Mobedi I, Morgan-Ryan UM, Thompson RC. Molecular and morphological characterization of *Echinococcus granulosus* of human and animal origin in Iran. Parasitology 2002; 125:367-373.
20. Rokni MB. Echinococcosis /hydatidosis in Iran. Iran J Parasitol. 2009; 4(2):1-16.