

Short Report

## Comparison of Dot-ELISA and Sandwich ELISA Diagnostic Tests in Detection of Human Hydatidosis

Mohammad Reza Siavashi<sup>\*1</sup>, Heshmatollah Taherkhani<sup>2</sup>, Kiumarsh Rezaei<sup>2</sup>, Mohammad Reza Razavi Deligani<sup>1</sup> and Mehdi Assmar<sup>1</sup>

<sup>1</sup>Dept. of Parasitology, the Pasteur Institute of Iran, Tehran; <sup>2</sup>Dept. of Parasitology, Hamadan University of Medical Sciences, Hamadan, Iran

Received 10 March 2004; revised 11 August 2004; accepted 5 September 2004

### ABSTRACT

Hydatidosis, caused by the larval stage of *Echinococcus granulosus*, is one of the most important zoonosis with worldwide distribution. As its diagnosis by clinical symptoms and scanning alone is difficult and confusing, we designed the present study to achieve a sensitive and simple diagnostic method for epidemiological studies. Sera (250 samples) were collected from 90 cases of hydatidosis proven by surgical operation, 80 patients with diseases other than hydatidosis and 80 healthy cases. The antigen (Ag) used was a crude hydatid fluid Ag obtained from lung and liver cysts of sheep slaughtered in Tehran abattoir (Iran). The result of dot-ELISA showed 100% sensitivity and 98.75% specificity. Positive and negative predictive values were 97.82% and 100% respectively. In the case of sandwich ELISA, the results were as follows: 92.22% sensitivity, 98.75% specificity, positive and negative predictive values: 97.64% and 95.75%, respectively. In both techniques, cross reaction with fasciolosis was observed for two cases. In conclusion, although these two tests had very similar results, dot-ELISA was more acceptable with respect to its higher sensitivity and simplicity in practice. *Iran. Biomed. J. 9 (2): 91-94, 2005*

**Keywords:** Human cystic hydatidosis, *Echinococcus granulosus*, Diagnosis

### INTRODUCTION

Human cystic hydatid disease (CHD) caused by the larval stage (hydatid cyst) of the dog tapeworm, *Echinococcus granulosus*, is a major infection with world wide distribution and variable geographical incidence [1]. Human infection is common in countries where sheep and cattle rearing constitute an important industry. As diagnosis of this disease by clinical symptoms and scanning alone is often difficult and confusing, some reliable and sensitive serological tests are required to corroborate the evidence reached. For the time being, specific diagnosis of CHD is based on immunological methods supplemented with radiological and ultrasound examinations. Diagnosis of the condition is important not only for detection of cases but also for surveillance of the disease in the community and also for monitoring

the impact of a control program for the disease in an area [2].

Complement fixation test (CFT) was the first immunological test used for serodiagnosis of CHD. Since then, a wide number of immunological tests have been developed for the detection of hydatid antibodies and of late hydatid antigens in the serum [3]. The hydatid anti-based serological tests include indirect haemagglutination (IHA), indirect immunofluorescence (IFA), immunoelectrophoresis, counter-current immuno electrophoresis (CIEP), radio-immunoassay (RIA) and ELISA. Development include enzyme-linked immunoelectrotransfer blots (EITB) [4], enzyme-linked immunoelectrodiffusion assay (ELIEDA) [5], time-resolved fluoroimmunoassay (TR-FLA) [6] and immunoblot [7]. The hydatid antigen-based sero-logical tests include mainly the ELISA [8, 9].

\*Corresponding Author;

Many of these assays with higher sensitivity and specificity require sophisticated equipment and trained technicians. Therefore, there is a need for an immunoassay to be simple and inexpensive. Also, its reagents or chemicals to be available and not requiring any heat-labile perishable reagents or chemicals. So we decided to evaluate the two latest and most popular techniques and choose one for routine laboratory work and seroepidemiological studies.

## MATERIALS AND METHODS

Sera (250 samples) were collected from 3 groups of people including 90 sera from patients with hydatidosis, proven by surgical operations from Kermanshah, Hamadan and Tehran General Hospitals (Iran), 80 sera from patients with the diseases other than hydatidosis and 80 sera from healthy blood donors referred to Hamadan Blood Transfusion Center (Iran) screened by a variety of standard imaging and paraclinical methods.

**Preparation of antigen.** Hydatid-infected livers and lungs of the sheep slaughtered in Tehran Abattoir (Iran) were collected and transported to the Parasitology Department of the Pasteur Institute of Iran (Tehran). There, the surface of the cysts was disinfected by iodine alcohol and the cyst fluid was aspirated under sterile condition. Then, 5 mM phenylmethylsulfonyl fluoride (PMSF, Sigma products, USA) as an anti-protease was added and mixed to make a suspension. The suspension was centrifuged at  $1,500 \times g$  at  $4^{\circ}\text{C}$  for 1 h to separate protoscolexes and other solid agents. The supernatant was used as the crude hydatid fluid antigen (CHF Ag) [10].

**ELISA.** This method was performed in 96-well immunolon essentially as described by Wen and Craig [11]. In brief, the wells were coated overnight at  $4^{\circ}\text{C}$  with  $100 \mu\text{l}$  CHF Ag [5] and after washing with 0.15 M PBS, pH 7.2 and 0.05% Tween 20, the wells were blocked in PBS containing 0.2 ml of 0.15 albumin and 0.3% Tween 20 for 1 h at room temperature. Serum samples dilution (1:20) in a final volume of 0.1 ml were added to PBS with 0.5% milk and 0.3% Tween 20 for 90 min at room temperature. After washing in washing buffer, the plate was incubated with mouse anti-human globulin IgG (Sigma, USA) with PBS for 1 h at room temperature. The plate was washed and then the secondary antibody, sheep anti-mouse IgG

phosphatase (Sigma, USA) was added at 1:500 for 1 h at room temperature. This was then developed in p-nitrophenylphosphate (pNP, Sigma, USA) and the absorbance was read at 450 nm after 20 minutes using an automatic micro plate reader.

**Dot-ELISA.** This method was developed as a modification of ELISA using nitrocellulose membrane as a carrier of protein as described by Rogan [12] and Romia [13] for diagnosis of hydatid disease. In brief, crude Ag was diluted 1:50 in PBS to a final concentration of  $20 \mu\text{g/ml}$  and dotted on PVDF strips (Roche Diagnostic, Germany). The strips were dried at  $4^{\circ}\text{C}$  overnight and blocked for 45 min in 0.1 molar TBST (10 mM of Tris base, 150 mM of NaCl and 0.5% of Tween 20) with 0.1 ml of 0.1% BSA and Tween 20 at room temperature. Test sera were diluted 1:10 in TBS solution and 1 ml of that was added to the strips and left for 30 min at room temperature. The strips were washed in TBST for 3 times (5 min for each time) before the conjugated anti-human IgG was added (1:800 diluted in TBS). After a further incubation period of 15 min at room temperature and humid atmosphere (inside a wet plate), the strips were again washed in TBTS for 3 times of 5 minutes before developing in 1 ml of a 60 ml TBS solution containing 30 mg DAB and  $6 \mu\text{l}$   $\text{H}_2\text{O}_2$  for 10 min. The reaction was stopped in PBS after 20 min.

## RESULTS

Complete analysis of our data showed that 90 cases of hydatidosis were in the age group of 19-80 years with the average of  $44.11 (\pm 1.5)$  years. Average age of 80 healthy cases was  $28.85 (\pm 0.85)$  years in the range of 18-46 years and in the 3<sup>rd</sup> group, 80 patients with diseases other than hydatidosis were in the age of 8-82 and average of  $36.86 (\pm 2.3)$  years. In the 3<sup>rd</sup> group, (Fig. 1) most of the patients had cancers and the lowest frequency belonged to appendicitis.

Table 1 shows all hydatidosis cases (1<sup>st</sup> group) are positive by dot-ELISA, but only 83 out of 90 cases (92.22%) were positive by ELISA. That shows superiority of dot-ELISA in p-value: 0.016. But in the case of the 2<sup>nd</sup> group (non-hydatidosis patients, which were expected negative for hydatidosis) we observed 2 false positive for both techniques.

Positive predictive value (PPV) of dot-ELISA was 97.82% (90 out of 92 cases) which had no significant difference with PPV of ELISA which

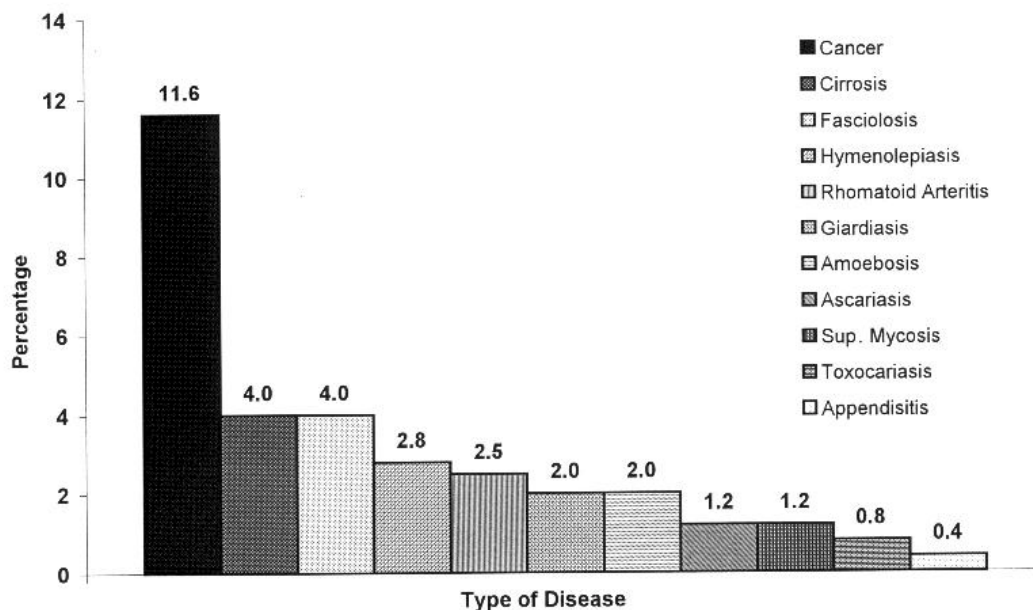


Fig. 1. Type of diseases other than hydatidosis used in control group.

was 97.64 (83 out of 85 cases). On the other hand, negative predictive value (NPV) of dot-ELISA was 100% (158 of 158 cases), which had a significant difference with ELISA with NPV of 95.75% (158 out of 165 cases).

## DISCUSSION

Immune response in hydatidosis, the basis of laboratory diagnosis, is quantitatively small and frequently insufficiently intense to be detected serologically. This has caused a constant search for increasingly sensitive techniques to detect very low antibody levels. To achieve this, several immunological methods have been evaluated in recent years [3-7]. In this study, using crude antigen of hydatid fluid, the sensitivity for the ELISA and dot-ELISA was 92.22% and 100%, respectively. The high sensitivity of dot-ELISA is due to that nitrocellulose paper can detect trace amounts of antibody. The specificity of both tests was 98.75%. These results showed that dot-ELISA is relatively better than

ELISA. The above amounts are comparable to Wang [14] that used antigen B (extracted from hydatid fluid) and showed that dot-ELISA was strongly positive in serologically confirmed patients. Also, Rogan *et al.* [12] have reported a sensitivity of 94% and specificity of 90.5% using dot-ELISA with Ag B as a field test for diagnosis of hydatid disease in the Turkana region, located in north-west of Kenya. This test was rapid and the result was obtained within 30 min of testing with 50  $\mu$ l of whole blood sample. In another study conducted in Egypt, Romia *et al.* [13] have reported a sensitivity of 88% and specificity of 96.9% by ELISA for demonstration of antibodies in 18 patients with hydatid disease and 32 blood donor controls. They have also evaluated dot-ELISA for detection of circulating hydatid antigen in the serum using anti-echinococcal hyperimmune rabbit sera. The sensitivity of the test however was relatively low (55.6%), which was attributed to the low volume of circulating antigen and/or formation of immune complexes, resulting in absence of free antigen in the serum.

Table 1. Comparison of sensitivity, specificity, positive and negative predictive values of ELISA and dot-ELISA in detection of human hydatidosis.

Type of test	Hydatidosis patient no. 90		Non-hydatidosis patient no. 80		Healthy cases no. 80		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.				
ELISA	83	7	2	78	0	80	92.22	97.64	97.64	95.75
Dot ELISA	90	0	2	78	0	80	100.0	97.72	97.82	100.0

PPV, positive predictive value; NPV, negative predictive value

The same studies were carried out by different researchers; Zhang and McManus [15] used crude antigen of hydatid fluid for dot-ELISA and with the titer of 1:400 reported a sensitivity of 92.3% and specificity of 89.9% for this test. In 1996, Abdel and Hady [16] compared three tests: ELISA, CIEP and IHA using crude and purified Ag. According to Abdel report, ELISA had the lowest cross-reaction with sensitivity of 68%, but in all tests similar to our study, crude Ag showed higher sensitivity than the purified one. In our study, we observed more sensitivity for ELISA and dot-ELISA than others (92.22%, 100%), respectively and specificity of 98.75% for both tests.

In 1987, Akhlaghi (personal communication) compared IHA with dot-ELISA using sheep hydatid fluid and showed the sensitivity of 86% and the specificity of 98% for dot-ELISA. In another study, Ghaffarifar *et al.* (personal communication) used Ag B of hydatid fluid in dot-ELISA and reported 100% sensitivity and 97.5% specificity. Both of these studies are comparable with our work in which we used crude Ag of sheep hydatid fluid and observed more reliable results by dot-ELISA.

In all of these studies, the cross-reactivity of hydatidosis was with fasciolosis [12, 13] which can be important in regions where both CHD and fasciolosis are endemic diseases, but in Iran, most fasciolosis cases are reported from Gilan province (north of Iran) where CHD is not a prevalent disease.

Dot-ELISA, as a test for field or poorly-equipped laboratories, offers many advantages; it is simple, rapid, sensitive, with minimal training and require any special equipment and the results can be observed visually by naked eye. Therefore, dot-ELISA can be recommended as a routine test in paraclinical laboratories and epidemiological studies.

## REFERENCES

1. Craig, P.S., Rogan, M.T. and Campos-Ponce, M. (2003) Echinococcosis: disease, detection and transmission. *Parasitology* 127 Suppl: S5-20.
2. Biffin, A.H., Jones, M.A. and Palmeri, S.R. (1993) Human hydatid disease: evaluation of ELISA for diagnosis, population screening and monitoring of control programs. *J. Med. Microbiol.* 39: 48-52.
3. Zarzosa, M.P., Orduna Domingo, A., Gutierrez, P., Alonso, P., Cuervo, M., Prado, A., Bratos, M.A., Garcia-Yuste, M., Ramos, G., Rodriguez, A. and Torros, A. (1999) Evaluation of six serological tests in diagnosis and postoperative control of pulmonary hydatid disease patients. *Diagn. Microbiol. Infect. Dis.* 35 (4): 255-262.
4. Ortona, E., Rigano, R., Margutti, P., Noralgiacono, S., Ioppolo, S., Vaccari, S., Barca, S., Buttari, B., Profuma, E., Teggi, A. and Siracusano, A. (2000) Native and recombinant antigens in the immuno-diagnosis of human cystic Echinococcosis. *Parasite Immunol.* 22 (11): 553-559.
5. Babba, H., Messedi, A., Masmoudi, S., Zribi, M., Grillot, R.I., Ambriose-Thomas, P., Beyrouiti, I. and Sahnoun, Y. (1994) Diagnosis of human hydatidosis: comparison between imaging and six serologic techniques. *Am. J. Trop. Med. Hyg.* 50: 64-68.
6. Aceti, A., Pennica, A., Teggi, A., Grilli, A., Caferro, M., Celestino, D., Leri, O., Sebastiani, A. and De Rosa, F. (1991) The serological diagnosis of human hydatid disease by time-resolved fluoroimmunoassay. *J. Infect.* 22: 135-141
7. Moosa, R.A. and Abdel-Hafez, S.K. (1994) Serodiagnosis and seroepidemiology of human unilocular hydatidosis. *Jordan. Parasitol. Res.* 80: 664-671.
8. Craig, P.S. and Nelson, G.S. (1984) The detection of circulating antigen in human hydatid disease. *Am. J. Trop. Med. Parasitol.* 78: 219-227.
9. Gottstein, B. (1984) An immunoassay for the detection of circulating antigens in human echinococcosis. *Am. J. Trop. Med. Hyg.* 33: 1105-1109.
10. Rogan, M.T. (1997) Analytical Parasitology, Springer-Verlag, Berlin, Germany, pp: 347.
11. Wen, H. and Craig, P.S. (1994) IgG subclass responses in human cystic and alveolar echinococcosis. *Am. J. Trop. Med. Hyg.* 51: 741-748.
12. Rogan, M.T., Craig, P.S., Zeyhle, E., Romig, T., Lubano, G.M. and Deshan, L. (1991) Evaluation of a rapid dot-ELISA as a field test for the diagnosis of cystic hydatid disease. *Trans. R. Soc. Trop. Med. Hyg.* 85: 773-777.
13. Romia, S.A., Yossef, M.E., Handoussa, A.E., Rizak, H.M. and Sallam, S.M. (1992) Dot-ELISA as a diagnostic test in hydatid disease. *J. Egypt. Soc. Parasitol.* 22: 603-610.
14. Wang, Y., Bradshaw, H., Rogan, M.T. and Craig, P.S. (2002) Rapid dot-ELISA for detection of specific antigens in the cyst fluid from human cases of cystic echinococcosis. *Ann. Trop. Med. Parasitol.* 96 (7): 691-694.
15. Zhang, L. H. McManus, D. P. (1996) Purification of N-terminal amino acid sequencing of *E. granulosus* antigen 5. *Parasit Immunology* dec; 18(12): 597-606
16. Abdel, A. Hady, A. L. (1996) Studies on most reactive purified antigen for immuno-diagnosis of hydatid disease. *J. Egypt. Soc. Parasitol.* 26(2) : 297-303.