Iranian J Parasitol: Vol.1, No.1, 2006, pp. 26-30

Evaluation of Dot-ELISA Method Using Excretory-Secretary Antigens of *Fasciola hepatica* in Laboratory Diagnosis of Human Fasciolosis

***MB Rokni [1], A Samani [1], J Massoud [1], M Babaei Nasr [2]**

[1]*Dept. of Medical Parasitology and Mycology, School of Public Health, and Institute of Public Health Research, Tehran University of Medical Sciences, Iran*
[2]*Center of Computer, School of Public Health and IPHR, Tehran University of Medical Sciences, Iran*

(Received 4 July 2006; accepted 12 Sep 2006)

Abstract

Fasciolosis diagnosis, due to low sensitivity of coprological diagnostic method has been challenging for a long period. In this study, Dot-ELISA, one of the simplest and the most sensitive tests in this regard, was evaluated using excretory-secretory antigens of *Fasciola hepatica* to diagnose human fasciolosis Three groups consisting of patients infected with fasciolosis (n= 95), patients with other parasitic diseases (n= 37) and healthy individuals (n= 40), were implicated in the test. All collected sera were tested by Dot-ELISA using excretory-secretory antigens. Optimal criteria were detected as 1.5 µg of antigen per dot, serum dilution of 1:320, and anti human IgG conjugate dilution of 1:500. The sensitivity, specificity, positive and negative predictive values were 96.8%, 96.1%, 96.8% and 96.1%, respectively. In conclusion, Dot-ELISA using excretory-secretory antigens could be regarded as a cheap, rapid, antigen and serum conservative diagnostic method in diagnosing fasciolosis.

Keywords: *Dot-ELISA, Excretory-secretory antigen,* Fasciola hepatica*, Diagnosis*

Introduction

Amongst the parasitic diseases, human fasciolosis, caused by the liver fluke, *Fasciola hepatica* or *F. gigantica* causes important damages to liver due to its natural residence in bile ducts (1). Not mention of human infection, animal infectivity, is considered of significance as well. The disease is transmitted to human by eating contaminated plants with infective metacercaria, which are derived from an intermediate molluscan host. Although in many laboratories, parasitological diagnosis is regarded as a routine procedure, but due to many obstacles it is not of valuable sensitivity (1). Many serological methods, thus far, have been challenged to diagnose human and animal fasciolosis, most of them vary in specificity and sensitivity due to differences in materials and methods, and of course, may owe to differences in the nature of the parasite, being utilized to prepare antigen. Of these methods, can be mentioned of haemaglu-tination (HA), indirect fluorescence antibody test (IFAT), immunoperoxydase (IP), counterelectrophoresis (CEP), enzyme-linked immunosorbent assay (ELISA) and Dot-ELISA (2, 3-11). The latter technique, due to simplicity, having the prospect to be im-

***Corresponding author:** Tel/ Fax: +98 21 88950184, E-mail: roknimoh@tums.ac.ir

planted in field trials, and so on, is regarded as an important method in its turn (2, 3, 5, 9, 11). In this process, even a small drop of serum can be utilized in the test.
The present study undertook to challenge Dot-ELISA technique in diagnosing of human and bovine fasciolosis, and excretory-secretory (E/ S) materials of *F. hepatica* was used as antigen.

Materials and Methods

Sera

Three groups consisting of patients infected with fasciolosis (n= 95), patients with some other parasitic diseases (n= 37) and healthy individuals (n= 40), were implicated in the test. Fasciolosis cases were diagnosed based on stool examination and ELISA test. Because northern Iran is one of the endemic areas of fasciolosis (15), it was not complicated to assemble infected sera. Non-fasciolosis serum cases were from patients infected with hydatidosis (n= 7), trichostrongylosis (n= 7), toxocariasis (n= 6), amoebiasis (n= 5), strongyloidiasis (n= 7) and malaria (n= 5) which were acquired from the School of Public Health, Tehran University of Medical Sciences (TUMS), Iran serum blood bank. The human's ethics committee at the School of Public Health, TUMS, approved the study.

Antigen preparation

Excretory/ secretory products (E/S) were prepared as described by Dalton and Heffernan (3). The concentration of each antigen preparation was measured using Bradford method (13). All antigens were preserved at -35 ºC until used.

Dot-ELISA

Dot-ELISA was conducted as described earlier (14). Briefly, 1.5 µg of *Fasciola* E/ S antigen was dotted on nitrocellulose membrane discs and allowed to be dried thoroughly. The discs were placed into flat bottom micro plate wells. Non-specific binding sites were blocked by addition 100 µl of tris buffer solution containing 0.5% Tween 20 (TBS/ T) to each well. Blocking solution was then aspirated off and antigen disks were washed by shaking (three times, 10 min each) with 0.05% Tween 20 (Riedel de Haen, AG, Seelze, Hanover, Germany) in TBS (vol/ vol). One hundred microliters of serum samples diluted 1: 320 in TBS/ T (after checkboard for serum dilution) was added to each disk and incubated for 45 min at room temperature. The serum samples were removed and washings were conducted as described above. The washing solution was removed and 100 µl of horseradish peroxidase-labeled goat anti-human IgG conjugate (Sigma, Chemical Co) diluted in TBS/T were added to each well and incubated for 45 min at room temperature. The optimum dilution of the conjugate was found to be 1: 500 by block titration of two-fold dilutions of the conjugate. The conjugate was removed and other washings were conducted as mentioned before. One hundred microliters of the substrate chromogen diamino 3, 3' benzidin tetrahydrocholoride (Sigma) was added to each well and incubated for 30 min at room temperature. The development of a deep brown colour dot on disks when compared with negative serum control was considered to be evidence of positivity. Colour development in controls was negligible or completely absent.

Statistical analysis

Standard diagnostic indices including sensitivity, specificity, positive and negative predictive values were calculated as described by Galen (15).

Results

Optimal criteria for conducting Dot-ELISA were detected as 1.5 µg of antigen per dot,

serum dilution of 1: 320, and antihuman IgG conjugate dilution of 1: 500. A serial dilution of 1: 10 to 1: 640 was used for sera to check the best output of diagnosis parameters; eventually the serum titer of 1:320 was found optimum to proceed the trial. One case of hydatidosis and two cases of healthy controls showed false positive reactions in this serum titer. Based on the cut-off value of 1: 320 for serum dilution, the sensitivity, specificity, positive and negative predictive values of the test were detected as 96.8%, 96.1%, 96.8% and 96.1%, respectively.

Discussion

Dot-ELISA has been considered as one of the valuable methods in diagnosis of different parasitological diseases including fasciolosis (3, 5, 9, 11), toxoplasmosis (4), shistosomiasis (17), hydatidosis (2) and cysticercosis (18).

As stated in the Result section, antibodies in the serum of one case of hydatidosis and two cases of healthy controls were reactive with *F. hepatica* E/ S antigen suggesting that an antigen in the *F. hepatica* E/ S shares epitope(s) with a hydatid. Moreover, polyparsitosis, when an individual may harbour two or three parasitic infections at the same time raises the possibility of cross-reaction.

Diagnostic parameters in this study showed promising feature, and based on the exclusive characteristics of Dot-ELISA mentioned earlier, it could be regarded as an authentic diagnostic test. Various re-searchers in the world have reported more or less approved this conclusion.

According to Dalimi *et al.* study, using *Fasciola gigantica* partially purified antigen, and with a 1: 800 sera dilution as the cut-off titer, the sensitivity of the Dot-ELISA test in diagnosis of human fasciolosis was 94.23% and the specificity was 99.36% (19).

Hassan and colleagues conducted Dot-ELISA using anti-*Fasciola* IgG isotypes. They reported that IgG2 and IgG4 demonstrated the highest specificity (> 99%), followed by IgG1 (90%) and the least specific test was obtained with detection of IgG (85%) (7). They concluded that detection of anti-*Fasciola* isotypes especially IgG4 was very specific for accurate diagnosis of fasciolosis. In another study conducted by Intapan *et al*., immunodominant antigens of an approximate molecular mass of 27 kDa (FG 27) were obtained from an excretory-secretory product of adult *Fasciola gigantica* by a simple continuous-elution method (5). A Dot-ELISA using this antigen showed the accuracy, sensitivity, specificity, and positive and negative predictive values as 98.2%, 100%, 97.4%, 76.9% and 100%, respectively (5). These values were a little bit more than our findings. Eventually, they concluded that Dot-ELISA was a specific, sensitive and easy to perform method for the rapid diagnosis of fasciolosis.

Shaheen *et al*. compared Dot-ELISA with micro-ELISA in diagnosing human fasciolosis using partially purified antigens from a species of *Fasciola* at 180 ng protein/ dot (2 microliters) and serum samples at 1: 20 dilution. They found that Dot-ELISA results completely agreed with those of micro-ELISA (9).

In conclusion, as we noted, the sensitivity, specificity, and speed of the Dot-ELISA may explain its exploitation in laboratory and field studies. Its ability to implement in field trials, little use of antigen and antibody, validity in screening tryouts and a lot of other benefits makes it as an authentic and applicable test in diagnosing human fasciolosis.

Acknowledgements

We express great appreciation to Miss Mojgan Aryaeipour, Neda Mirsepahi and Mr Ali Rahimi from the Tehran University of Medical Sciences, Iran, for their sincere cooperation. This work was supported by the Institute of Public Health Research, School of Public Health, TUMS, Iran.

References

1. Hillyer GV. Immunodiagnosis of human and animal fasciolosis. In: *Fasciolosis*. Ed, JP Dalton. CABI Publishing, Oxon, Wallingford, UK; 1998. p.p 435-48.
2. Hadighi R, Mirhadi F, Rokni MB. Evaluation of a dot-ELISA for the serodiagnosis of human hydatid disease. Pak J Med Sci. 2003; 19: 268-71.
3. Hassan MM, Matar-nowafy MA, Saleh A *et al*. Dot ELISA for measuring anti-*Fasciola* IgG isotype among patients with Fascioliasis. J Egypt Soc Parasitol. 2002; 32: 571-8.
4. Hillyer GV, Sanchez Z, De Leon D. Immunodiagnosis of bovine fascioliasis by enzyme-linked immunosorbent assay and immunoprecipitation methods. J Parasitol. 1985; 71: 449-54.
5. Intapan PM, Maleewony W, Nateeworanart S *et al*. Immunodiagnosis of human fasciolasis using an antigen of *Fasciola gigantica* adult worm with the molecular mass of 27 kDa by dot-ELISA. Southeast Asian J Trop Med Public Health. 2003; 34(4): 713-17.
6. Leevieux D, Levieux A, Mage C, Venien A. Early immunodiagnosis of bovine fascioliasis using the specific antigen f2 in a passive hemagglutination test. Vet Parasitol. 1992; 44: 77- 86.
7. Levieux D, Levieux A. Early immunodiagnosis of caprine fasciolosis using the specific f2 antigen in a passive hemagglutination test. Vet Parasitol. 1994; 53: 59-66.
8. Rokni MB, Massod J, Parkinson M, O'Neill S, Dalton JP. Diagnosis of human fasciolosis in the Gilan Province of Northern Iran: application of cathepsin L-ELISA. Diag Microbio Infec Dis. 2002; 44(2): 175-79.
9. Shaheem HI, Kamal KA, Farid Z, Mansour N, Boctor FN, Woody JN. Dot- enzyme- linkd- immunosorbent assay (Dot ELISA) for the rapid diagnosis of human fascioliasis. J Parasitol. 1989; 75(5): 549-52.
10. Tkalcevic J, Brandon MR, Meeusen ENT. *Fasciola hepatica*: rapid switching of stage-specific antigen expression after infection. Parasit Immunol. 1996; 18: 139-47.
11. Zimmerman GL, Nelson MJ. Diagnosis of ovin fascioliasis by Dot ELISA: a rapid microdiagnostic technique. Am J Vet Res. 1985; 40(7): 1513-15.
12. Dalton JP, Heffernan M. Thiol protease released in vitro by *Fasciola hepatica*. Mol Bio Para. 1989; 35: 161-66.
13. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantitive protein utilizing the principle of protein-dye binding. Anal Biochem. 1976; 72: 248-54.
14. Pappas MG. Recent Application of Dot- ELISA in immunoparasitology. Vet Parasitol. 1988; 29:105-29.
15. Galen RS. Predictive values and efficiency of laboratory testing. Pediat J Clin North Am. 1980; 27: 861-69.

16. Elsaid MM, Bahia M, Machado G *et al*. Diagnosis of human toxoplasmosis by a dot enzyme linkimmunosorbent assay. Rev inst Med Trop Saopaulo. 1995; 37: 117-22.
17. Liu YS, Du WP, Wu ZX. Dot-immunogold- silver staining in the diagnosis of cysticercosis. Int J Parasitol. 1996; 26(1): 127-9.
18. Schaefer KV, Weind T, Janitschke K. Comparison of three Dot- ELLISA techniques in diagnosing S*chisto-soma haematobium* infection. East Afr Med J. 1995; 72(12): 809-12.
19. Dalimi A, Hadighi R, Madani R. Partially purified fraction (PPF) antigen from adult *Fasciola gigantica* for the serodiagnosis of human fasciolosis using Dot-ELISA technique. Ann Saudi Med. 2004; 24: 18-20.