Comparison of ELISA and Agar Gel Diffusion Techniques in Diagnosis of Human Hydatidosis

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Summary

The comparative study between ELISA and agar gel diffusion (AGD) tests was conducted on 105 human samples obtained from 37 patients with surgically confirmed hydatidosis, 47 apparently healthy individuals, and 21 persons with other parasitic infections. Sheep hydatid fluid was dialyzed against PBS and concentrated by evaporation used as antigen. The crude antigen was used in ELISA and AGD for determining antibody production in sera taken from patients with hydatid and other parasitic infections. The sensitivity of 86.5% and 73 % were estimated for ELISA and AGD, respectively while the specificity of both tests was 92.6%. False negative was observed in 13.5% and 27% of confirmed hydatidosis for ELISA and AGD respectively.

Key words: diagnosis, human hydatidosis, ELISA, AGD, antigen

Introduction

Hydatidosis is a zoonotic disease, which caused by the larval stage of tapeworm genus Echinococcus in man and animals. The adult Echinococcus is a short worm that infects the small intestine of the canine or feline hosts. Man acquires hydatidosis by the ingestion of Echinococcus eggs via direct contact with dogs or from contaminated environment (Babba *et al* 1994, Rickard 1979). Hydatid disease is harmful for human that influenced by cyst size and organ location.

It is well known that the antibody response in the host plays an important role in Echinococcus infections. Fluid leaking from *E.granolosus* cyst into systemic circulation, have shown that IgA, IgM, IgG and IgE can be detected in hydatid infected patient sera (Matossian 1972 & 1977). Numerous studies have been carried out to developing serologic and immunodiagnostic tests for hydatidosis in man. Sensitive and reliable serologic techniques are necessary to confirm the diagnosis. Based on the detection of humoral responses of the host against parasite some serological methods employed for the diagnosis of hydatidosis disease including complement fixation test, indirect haemagglutination antibody, indirect fluorescent antibody, immunoelectrophoresis and ELISA (Kagan 1968, Lui *et al* 1992). Immunological diagnosis of hydatid infection in human based on the identification of precipitating band formed in AGD and immnunoelectrophoresis by crude and specific hydatid antigens and sera from *E.granolosus* infected patients have given results with positively rate of 57-92% (Bombardieri *et al* 1974). The sensitivity reported for the ELISA tests by previous authors (Kaddah 1992, Njeruh *et al* 1989) were 85-92%.

The aim of this study was to evaluate the sensitivity and specificity of AGD test by use of crude antigen in diagnosis of human hydatidosis and comparison of the results with ELISA.

Materials and Methods

Sera. Serum samples were obtained from 37 patients with hydatidosis confirmed by surgery, 47 apparently healthy individuals and 21 patients with other parasitic infections (Shiraz hospital and Hygiene faculty of Tehran, Uni.).

Antigens. Hydatid cyst fluid aspirated aseptically from fertile sheep liver or lung cysts in slaughterhouse was used as the source of antigen. The fluid was clarified by centrifugation and dialyzed against PBS (pH7.2) at 4°C for overnight. It was concentrated 3-fold by evaporation and the protein

concentration determined by Lowry method. Sodium azide (0.1%) was added to antigen and stored at -20°C in 5ml aliquots until used.

ELISA assay. Antigen was diluted in coating buffer (carbonate buffer, pH9.6) to a protein concentration of 10.3µg/ml and was added (100µl/well) to microtiter plates (Maxisorb, Nunc). The plates were incubated in a humid chamber at 4°C for overnight. The wells were washed with PBS, blocking buffer (PBS containing Tween 20, pH7.4) was added. The plates were incubated for 30min at 37°C. 100µl of 1/200 diluted sera were added then plates incubated for 1.5h at 37°C. After washing conjuogate (anti-human IgG phosohatase, 1:1000, Sigma), substrate (1mg/ml pNpp) and stopping solution (NaOH, 4N) were added to each plate. Optical density (OD) was read at 405 nm, cut-off was calculated based on mean of OD plus three standard deviations of negative control sera and the results were interpreted according to Iacona *et al* 1980, Kaddah *et al* 1992, Hamdy *et al* 1997). Moreover, an ELISA kit, which donated by Dr. Deplazes (Zurich) was used as standard.

Agar Gel Diffusion. AGD test was carried out in Petri dishes of 10cm in diameter. Each plate was filled with 20ml of 1:100 purified agar (Special Agar Noble, Diffco) in 8% NaCl, pH7.2. The wells were cut by gel-cutter, then antigens and different human sera were added into central and peripheral wells (80µl/well), respectively. The plates were observed 24-48h for line precipitation (Bombardieri 1974).

Results

The results of ELISA and AGD tests in patients with hydatidosis, healthy individuals and other parasitic infections were shown in table 1. From 37 patients with hydatid cyst 5 (13.5 %) and 10 (27%) gave negative reactions by ELISA and AGD, respectively. The sera of 47 apparently healthy individuals gave negative results in both tests. In patients suffering from other infections (toxocariasis and facsioliasis) only 5 of them gave positive reactions ELISA and AGD. By attention to these data and analysis of them,

the sensitivity were 32 (86.5%) and 27 (73%) for ELISA and AGD, respectively. The specificity was 63 (92.6%) for both tests.

The cut-off ELISA assay was calculated according to O.D. of negative sera plus 3 S.D of control sera. The 0.3 and more was considered as positive. Of 37 sera from patients with hydatid cyst 27 (73%) gave positive reactions by standard ELISA kit and the results of non-infected individuals and those suffering from other infections were the same as our ELISA.

Table1. *Results of ELISA and AGD tests in patients with hydatidosis, healthy individuals and other parasitic infections*

Factors		Positive reaction		Negative reaction		Sensetivity		Specificity	
Type of serum	No. serum	ELISA	AGD	ELISA	AGD	ELISA	AGD	ELISA	AGD
Hydatidosis	37	32	27	5	10	86.5	73		
Healthy individual	47	0	0	47	47			92.6	92.6
Other diseases	21	5	5	16	16				
Total	105	37	32	68	73				

Discussion

The results of the AGD and ELISA tests described in this paper indicate that crude hydatid fluid can usefully apply in serological diagnosis, as an antigen. In this study, we have determined that the antigen gave a sensitivity of 86.5%, which is agreement (85%-92%) with other reports (Lui *et al* 1992, Njeruh *et al* 1989, Navarrete *et al* 1995). The specificity (92.6%) is the same as previous studies (Bombardieri & Ggiordano 1974, Kaddah *et al* 1992).

The precipitation pattern of crude hydatid fluids in AGD was different. It seems that the differences are due to different stage and viability of cyst and also mixed infections (*e.g* fascioliasis and toxocariasis). According to previous reports, hydatid cyst fluid has common antigens to other parasites such as Taenia, Schistosoma, Fasciola and Filaria (Kagan 1968, Bombardieri

& Ggiordano 1974). The serum of all normal persons gave negative result in AGD and ELISA and cross-reactions were the same in both. Bivo *et al* (1992), Babba *et al* (1994) and Rickard (1979) demonstrated that the ELISA was able to detect hydatid antibodies either by purified or crude hydatid fluid antigens. For screening and epidemiological study of hydatid disease the sensitivity and specificity of ELISA could be comparable to AGD test (Bombardieri & Ggiordano 1974, Navarrete *et al* 1995).

Since the ELISA has not routinely carried out in all laboratories, because of its equipment, and a reliable test has not established for definitive diagnosis of all cases yet, AGD is suggested for the detection of echinococcal antibodies in hydatidosis. In conclusion, according to this and other comparative studies the AGD is a suitable test for diagnosis of hydatid disease and screening of serum samples in epidemiological studies in high risk population.

References

Babba, H., Messedi, A. (1994). Diagnosis of human hydatidosis: Comparison between imagery and six serologic techniques. *American Journal of Tropical Medicine and Hygiene* 50:64-68.

Bombardieri, S., Ggiordano, F. (1974). An evaluation of an agar gel diffusion test with crude and purified antigens in the diagnosis of hydatid disease. *Bulletin of World Health Organization* 51:525.

Hamdy, A. E., Bakr, M. E., Nassef, N. E. and Elkersh, W. M. (1997). Serodiagnosis of hydatid disease. *Journal of Egypt Society Parasitol.* 27:67-73.

Iacona, A., Pini, C. and Vicari, G. (1980). Enzyme linked immuno-sorbent assay (ELISA) in serodiagnosis of hydatid disease. *American Journal of Tropical Medicine and Hygiene* 29:95-102.

Kagan, I. G. (1968). Review of serological tests for the diagnosis of hydatid disease. *Bulletin of World Health Organization* 39:25-37.

Kaddah , M. H., Maher, K. M., Hassanein, H. I., Farrag, A. T., Shaker, Z. A. and Khalafallah, A. M. (1992). Evaluation of different immonudiagnostic techniques for diagnosis of hydatidosis in Egypt. *Journal of Egypt Society Parasitol.* 22:653-65.

Liu, D., Lightowlers, M. W. and Rickard, M. D. (1992). Evaluation of monoclonal antibody-based compassion ELISA for the diagnosis of human hydatidosis. *Parasitology* 104:357-361.

Matossian, R. M. (1972). The specific immonoglobolin in hydatid disease. *Immunology* 22:423-430.

Matossian, R. M. (1997). The immunological diagnosis of human hydatid disease. *Transaction of Royal Society at Tropical Medicine and Hygiene* 71:101-104.

Navarrete, N., Jersic, M. I. and Denis, R. (1995). Comparison of three techniques for serological diagnosis of human hydatidosis *Bulletin of Children Parasitology* 50:97-100.

Njeruh, F. M., Okelo, G. B. and Gathuma, J. M. (1989). Usefulness of indirect heamagglutination (IHA) and ELISA in the diagnosis of human hydatidosis. *East African Medical Journal* 66:310-314.

Rickard, M. D. (1979). The immunological diagnosis of hydatid disease. *Australian Veterinary Journal* 55:99-104.