

Evaluation of Two ELISA and Two Indirect Hemagglutination Tests for Serodiagnosis of Pulmonary Hydatid Disease

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Abstract: To establish a definite diagnosis for pulmonary hydatid disease, combination of radiology and serology is useful. In this study, 19 preoperative sera from patients with surgically confirmed pulmonary hydatidosis, 40 sera from patients with other parasitosis and pulmonary diseases, and 20 sera from healthy donors were evaluated using 4 different serological tests, i.e., the commercial ELISA (ELISA-kit) test, the ELISA (ELISA-lab) test prepared in our laboratory, the commercial indirect hemagglutination assay kit (IHA-kit) test, and the IHA test using sensitized sheep red blood cells with tannic acid (IHA-TA). The ELISA-kit was the most sensitive (84.2%) and the most specific test (100.0%). The ELISA-kit also demonstrated the highest positive (100.0%) and negative (95.2%) predictive values. The sensitivity of the ELISA-lab test, that we prepared, was found to be 73.6%, whereas the IHA-kit test and the IHA-TA test were found to be 73.6% and 68.4%, respectively. The specificity of these tests was 96.6%, 98.3%, and 83.3%, respectively. When all 4 tests were assessed together, it was found that the sensitivity had risen to 94.7%. When the ELISA-kit was assessed with the IHA-kit and IHA-TA together, it was found that the sensitivity was 89.5% and 84.2%, respectively. Likewise, the combination of the ELISA-lab and IHA-kit or IHA-TA allowed us to achieve a sensitivity of 84.2% in cases of pulmonary echinococcosis. In conclusion, the diagnosis would be imminent if least 2 tests were applied together.

Key words: *Echinococcus granulosus*, pulmonary hydatid disease, ELISA, IHA, serodiagnosis

Human primary hydatid is most frequently seen in the liver and the lung. Radiology and serology should be assessed together to establish a definite diagnosis for pulmonary hydatid disease [1,2]. There are studies reporting that the antibody response is lower in lung hydatid cysts compared with liver hydatid cysts; and serologic tests have 80-90% sensitivity in liver hydatid cysts and 50-56% in pulmonary hydatid cysts [3-5]. The best result in the diagnosis of pulmonary hydatid cysts is obtained by collective use of radiology and serology. Nevertheless, serology is more specific but less sensitive than imagery [6]. Among the serologic tests, IgG ELISA and indirect hemagglutination (IHA) are 2 important tests often used in diagnosis and in monitoring the disease in the postoperative period [7-9]. In this study, 4 different serologic tests were applied to the sera of a group of patients with pulmonary hydatid disease, and the sensitivity, the specificity, positive and negative predictive values of the tests were assessed.

The operation results were taken as the golden standard, and the sensitivity and the specificity of the tests were calculated ac-

cordingly [2,9-11]. Patients' sera were separated so as to perform 4 tests. All sera were frozen and kept -80°C in aliquots until use.

Two ELISA and 2 IHA tests were performed for all patient sera. ELISA-lab and IHA-lab were prepared in our laboratory using hydatid cystic fluid (HCF) from fertile cysts of sheep liver. The antigen was prepared with a pool of HCF centrifuged at 15,000 g for 30 min. The supernatant was used as crude antigen. The commercial Hydatidosis IgG ELISA (Vircell SL, Granada, Spain) test was employed according to the manufacturer's instructions. Each test reaction was read spectrophotometrically at 405 nm. Cut-off optical density was calculated in cut-off control serum for IgG. Antibody index (AI) was calculated as follows: $AI = \text{sample OD} / \text{cut-off OD} \times 10$. AI greater than 11 was accepted as seropositive, and values lower than 9 were considered as seronegative. Values between 9 and 11 were accepted as borderline and the test was repeated for all these sera. ELISA was prepared by the method of Coltorti et al. [12]. The readings were taken at 410 nm. The serum dilutions that resulted in an absorbance at least twice the mean absorbance of wells containing negative control samples were considered positive. Thus, 1 : 160 and higher dilutions was accepted as positive [11]. The commercial Hydatidosis IHA (Hydatidose, Fumouze Laboratoires, France) test was employed according to the manufacturer's in-

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structions. A titer of $\geq 1 : 320$ was evaluated as positive. The fourth test we prepared in our own laboratory using HCF was the IHA test where erythrocytes were sensitized with tannic acid [13]. One hundred μl of the test or control sera diluted 1 : 32 and above in 1% inactivated rabbit serum were placed in each well. Next, each well was filled with 50 μl red blood cells. After 2 hr, serological reactivity was detected in all sera. A titer of $\geq 1 : 32$ was accepted as positive [13].

The sensitivity, specificity, positive and negative predictive values of each test was given. To compare sensitivity and specificity of each test, we used receiver-operating characteristic (ROC) curve of each test and presented area under curve (AUC). The area under the ROC curve for the ELISA-kit, ELISA-lab, IHA-kit, and IHA-TA was 0.921, 0.855, 0.863, and 0.828, respectively.

In the present study, operation results of the patients were accepted as the golden standard. Accordingly, when assessed with respect to pulmonary hydatid cysts, 12 of the 19 patient sera, which had definitely been determined to be positive, were found to be positive with all the tests. Six of the remaining sera were found to be positive with at least 1 test, whereas the seventh serum was not positive with any of the tests. Thus, when all 4 tests were assessed together, the sensitivity had risen to 94.7%. The positive and negative predictive values of the tests were over 81.2%. Specificity, sensitivity, and positive and negative predictive values are shown in Table 1. Furthermore, when we used ELISA and IHA tests together, the sensitivity increased markedly. When ELISA-kit was assessed with IHA-kit and IHA-TA together, the sensitivity was 89.5% and 84.2%, respectively. While ELISA-lab and IHA-kit or IHA-TA was used together, the sensitivity had risen from 73.6% to 84.2%. In addition, we determined 18 of the 19 positive patients when we used all 4 tests together, and the sensitivity reached 94.7%.

Using 40 sera from parasitosis and other pulmonary illnesses and 20 healthy subject sera as controls, specificity results of

Table 1. Serological test validity in diagnosis of pulmonary hydatidosis

	ELISA-kit	ELISA-lab	IHA-kit	IHA-TA
Sensitivity	84.2 (16/19)	73.6 (14/19)	73.6 (14/19)	68.4 (13/19)
Specificity	100.0 (60/60)	96.6 (58/60)	98.3 (59/60)	95.0 (57/60)
Positive predictive value	100.0 (16/16)	87.5 (14/16)	93.3 (14/15)	81.2 (13/16)
Negative predictive value	95.2 (60/63)	92.0 (58/63)	92.1 (59/64)	90.4 (57/63)

The results are in percentage and the number of patient.

The presence or absence of pulmonary hydatid cysts of the patients has been definitely proven operatively.

the tests were obtained. Of the 60 definitely negative patients, 56 were found to be negative in all the tests (Table 2). Of the remaining 4 patients, 1 patient with bronchopulmonary cancer was determined to be false positive in IHA-TA. Likewise, 1 patient with a parasitic disease (taeniasis saginata) was false positive in ELISA-lab. The last 2 patients (1 pulmonary infection, 1 healthy subject) were determined to be false positive in 2 tests (Table 2). Among the 4 serologic tests used in hydatidosis diagnosis, we found that the IHA-TA test gave the largest number of false positives.

Many studies have been carried out concerning immunoserologic tests of hydatidosis. These include studies reporting that the sensitivity of IgG ELISA is excellent [2,6,11,14,15]. Wattal et al. [11] found that sensitivity of IgG ELISA in patients with pulmonary hydatidosis was 100%. Sbihi et al. [16] assessed patients with *E. granulosus* having different localization by means of 4 different tests (latex agglutination, immunoelectrophoresis, and specific IgE and IgG ELISA), and they also found the sensitivity of IgG ELISA to be 96.5%. In their study, they conducted with 79 patients who were surgically proven to have pulmonary hydatid cysts. Zarzosa et al. [9] showed that the most sensitive test was specific IgG ELISA (83.5%). So as to obtain higher sensitivity, at least 2 serologic tests are used together in many laboratories. In studies carried out on Australian children, Rebhandl et al. [10] stated that they obtained successful sensitivity results (71%) by using new test combinations. Force et al. [8] made use of 8 different serologic tests (IgG, IgA, IgE ELISA, latex agglutination, IHA, total IgE, radioallergosorbent test (RAST), and immunoelectrophoresis) for the postoperative diagnosis of cystic disease associated with *E. granulosus* in 131 patients. They reported that IgG ELISA, IHA, and IgA ELISA exhibited a sensitivity of 81% when used together. In our study, we determined

Table 2. False positive results in serological tests in patients with other parasitosis and lung illnesses, and in healthy subjects

	No. sera	No. positive sera	ELISA-kit No. positive	ELISA-lab No. positive	IHA-kit No. positive	IHA-TA No. positive
Other parasitic disease	15	1	-	1	-	-
Pulmonary infections	10	1	-	-	1	1
Bronchopulmonary cancer	10	1	-	-	-	1
Pulmonary tuberculosis	5	-	-	-	-	-
Healthy subject	20	1	-	1	-	1
Total	60	4	-	2	1	3

18 of the 19 positive patients by using 2 ELISA tests and 2 IHA tests together, and found that using these tests together increased the sensitivity up to 94.7%.

Among the diseases causing cross reactions, parasitic diseases other than echinococcosis take the first place, followed by cirrhosis and liver and pulmonary malignancies [2,3]. Force et al. [8] demonstrated that many serologic tests led by IgE and IgA ELISA might be responsible for nonspecific reactions. In our study, we observed 1 patient with bronchopulmonary cancer false positive in 1 test, another patient with pneumonia false positive in 2 tests, and another patient with taeniasis false positive in 1 test.

In conclusion, we have established that serologic tests are valuable in hydatid cyst cases and that it would be appropriate to use these tests together with radiology for diagnosis and post-operative follow-up of the disease. We believe that if at least 2 serologic tests are applied, sensitivity in the diagnosis will reach higher values.

REFERENCES

- Schantz PM, Gottstein B. Echinococcosis (hydatidosis). In Walls KW, Schantz PM, eds, *Immunodiagnosis of Parasitic Diseases*. Vol. 1. Orlando, Florida, USA. Academic Press, Inc. 1986, p 69-107.
- Ramos G, Orduña A, Garcia-Yuste M. Hydatid cyst of the lung: Diagnosis and treatment. *World J Surg* 2001; 25: 46-57.
- Gottstein B. Molecular and immunological diagnosis of echinococcosis. *Clin Microbiol Rev* 1992; 5: 248-261.
- Craig PS, Nelson GS. The detection of circulating antigen in human hydatid disease. *Ann Trop Med Parasitol* 1984; 78: 219-227.
- Iacona A, Pini C, Vicari G. Enzyme-linked immunosorbent assay (ELISA) in the serodiagnosis of hydatid disease. *Am J Trop Med Hyg* 1980; 29: 95-102.
- Babba H, Messedi A, Masmoudi S, Zribi M, Grillot R, Ambriose-Thomas P, Beyrouti I, Sahnoun Y. Diagnosis of human hydatidosis: Comparison between imagery and six serologic techniques. *Am J Trop Med Hyg* 1994; 50: 64-68.
- Felgner P. Antibody activity in stick-ELISA as compared to other quantitative immunological tests in sera of echinococcosis cases. *Tropenmed Parasitol* 1978; 29: 417-422.
- Force L, Torres JM, Carrillo A, Buscá J. Evaluation of eight serological tests in the diagnosis of human echinococcosis and follow-up. *Clin Infect Dis* 1992; 15: 473-480.
- Zarzosa MP, Orduna Domingo A, Gutierrez P, Alonso P, Cuervo M, Prado A, Bratos MA, Garcia-Yuste M, Ramos G, Rodriguez Torres A. Evaluation of six serological tests in diagnosis and postoperative control of pulmonary hydatid disease patients. *Diagn Microbiol Infect Dis* 1999; 35: 255-262.
- Rebhandl W, Turnbull J, Felberbauer Fx, Tasci E, Puig S, Auer H, Paya K, Kluth D, Tasci O, Horcher E. Pulmonary echinococcosis (hydatidosis) in children: Results of surgical treatment. *Pediatr Pulmonol* 1999; 27: 336-340.
- Wattal C, Malla N, Khan IA, Agarwal SC. Comparative evaluation of enzyme-linked immunosorbent assay for the diagnosis of pulmonary echinococcosis. *J Clin Microbiol* 1986; 24: 41-46.
- Coltorti EA. Standardization and evaluation of an enzyme immunoassay as a screening test for the seroepidemiology of human hydatidosis. *Am J Trop Med Hyg* 1986; 35: 1000-1005.
- Varela-Díaz VM, López-Lemes Mh, Prezioso U, Coltorti EA, Yarzabal LA. Evaluation of four variants of the indirect hemagglutination test for human hydatidosis. *Am J Trop Med Hyg* 1975; 24: 304-311.
- Hira PR, Bahr GM, Shweiki HM, Behbehani K. An enzyme-linked immunosorbent assay using an arc 5 antigen for the diagnosis of cystic hydatid disease. *Ann Trop Med Parasitol* 1990; 84: 157-162.
- Knobloch J, Lederer I, Mannweiler E. Species-specific immunodiagnosis of human echinococcosis with crude antigens. *Eur J Clin Microbiol* 1984; 3: 554-555.
- Sbihi Y, Rmiqui A, Rodriguez-Cabezas MN, Orduña A, Rodriguez-Torres A, Osuna A. Comparative sensitivity of six serological tests and diagnostic value of ELISA using purified antigen in hydatidosis. *J Clin Lab Anal* 2001; 15: 14-18.