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IMMUNODIAGNOSIS OF HUMAN CYSTICERCOSIS (*TAENIA SOLIUM*): A FIELD COMPARISON OF AN ANTIBODY-ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA), AN ANTIGEN-ELISA, AND AN ENZYME-LINKED IMMUNOELECTROTRANSFER BLOT (EITB) ASSAY IN PERU

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Abstract. We compared results of an enzyme-linked immunosorbent assay (ELISA) and an enzyme-linked immmunoelectrotransfer blot (EITB) assay for the diagnosis of cysticercosis in sera and cerebrospinal fluid (CSF). Sera from 34 patients with confirmed cysticercosis were tested by both ELISA and EITB assays. Cerebrospinal fluid from some of these patients was also tested by ELISA for the presence of antibody (AB-ELISA) (n = 21) and antigen (AG-ELISA) (n = 15). Specificity in sera was examined by testing 51 serum samples from Bangladesh, where cysticercosis is not endemic. Cross-reactivity was evaluated in sera from patients with *Echinococcus granulosus* (hydatid) and *Hymenolepis nana* infections. Sensitivity in detecting cysticercosis in sera was 94% by EITB and 65% by AB-ELISA ($P < 0.01$). Sensitivities in the CSF tested by EITB, AB-ELISA, and AG-ELISA were 86%, 62%, and 67%, respectively. The specificity of the EITB was 100%, while that of AB-ELISA was 63% ($P < 0.01$). Cross-reactions occurred in the AB-ELISA with 11% and 20% of sera from hydatid and *H. nana* patients, respectively. Our results demonstrate that the EITB is the best assay available for the diagnosis of cysticercosis in both sera and CSF.

Cysticercosis, the disease produced by the larval stage of *Taenia solium*, is present worldwide. High rates of infection are found in India, Indonesia, Africa, Mexico, Peru, and other South American countries.[1-3] In both Peru and Mexico, cysticercosis accounts for over 10% of neurologic hospital admissions.[3]

Cysticercosis is frequently misdiagnosed because of its highly variable presentation. Computed tomography scan and magnetic resonance imaging can be helpful in diagnosing neurocysticercosis, but their use in developing countries is limited because of their high cost and limited availability.[4]

Three new serologic techniques have been reported to be useful in the diagnosis of cysticercosis: an enzyme-linked immunosorbent assay (ELISA) using a crude antigen for the detection of antibodies to *T. solium* (AB-ELISA) in serum or cerebrospinal fluid (CSF),[5] an antigen-detection ELISA limited to the CSF (AG-ELISA),[6] and an enzyme-linked immunoelectrotransfer blot (EITB) assay used to detect antibodies in the serum or CSF.[7]

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We conducted a prospective study to provide a realistic field trial for these three assays and to compare their diagnostic sensitivity and specificity. Sera and CSF samples were obtained from both tissue-confirmed cases of neurocysticercosis and from individuals who were infected with *T. solium* or *T. saginata* tapeworms. We also determined the rates of cross-reactivity with sera from patients with *Echinococcus granulosus* and *Hymenolepis nana* infection.

MATERIALS AND METHODS

Human sera and CSF

Serum and CSF specimens were obtained from tissue-confirmed cases of neurocysticercosis. All specimens were coded and tested in a blinded fashion. Duplicate specimens from a portion of the patients were sent to the Parasitic Diseases Branch (PDB) of the Centers for Disease Control (CDC) for quality control testing.

Clinical classification

The following definitions were used for clinical classification of the study participants: 1 = confirmed cysticercosis, patients in whom cysticercosis was confirmed by means of a biopsy, surgery, or presence of ocular cysticercus (n = 34); 2 = negative controls, persons from countries where cysticercosis is not normally transmitted (n = 11 from the United States and 51 from Bangladesh); and 3 = taeniasis patients, persons who eliminated a tapeworm or proglottides identified as *T. saginata* (n = 4), *T. solium* (n = 14), or *Diphyllobothrium* species (n = 2).

Antibody detection by ELISA (AB-ELISA)

Whole *T. solium* cysticerci were excised from the skeletal muscle of naturally infected pigs. Ten grams of the larvae were washed in 30 ml of phosphate-buffered saline (PBS) (0.137 M NaCl, 1.47 mM KH_2PO_4, 10.9 mM Na_2HPO_4, 2.7 mM KCl, pH 7.4) containing 0.25 M phenylmethylsulfonyl fluoride as a protease inhibitor. The larvae were homogenized in this solution at 4°C in a glass tissue homogenizer. The homogenate was sonicated three times (3 min/pulse at 20 kHz) on ice with 60-sec cooling intervals. The sonicate was centrifuged at 25,000 *g* in a SA-600 rotor (Dupont, Wilmington, DE) for 30 min at 4°C, and the supernatant was used directly as the crude antigen preparation.

The antigen obtained was diluted in carbonate buffer (pH 9.6) to a concentration of 1 μg/ml. The wells of microtiter plates (Immulon 1; Dynatech, Alexandria, VA) were coated with antigen by overnight incubation at 4°C. The plates were blocked with 5% nonfat milk in PBS with 1% Tween-20, and incubated at 37°C for 1 hr. Serum was diluted 1:2,500 with PBS containing 0.05% Tween 20 (PBS-Tween). After the samples were washed five times with PBS-Tween, serum was added to all wells, and the plates were incubated at room temperature for 2 hr. The plates were then washed five times, 100 μl of a 1:2,000 dilution of peroxidase-conjugated goat anti-human antibody (Kirkergaard and Perry, Gaithersburg, MD) was added to each well, and the plates were incubated for 1 hr at room temperature. The plates were then washed five times, and 100 μl of *o*-phenylenediamine peroxidase substrate (Sigma, St. Louis, MO) was added to each well. After a 15-min incubation, 100 μl of 0.1 M sulfuric acid was added to each well to stop the reaction. Optical density (OD) values at 492 nm were determined with a Multiscan (Flow Laboratories, McLean, VA) ELISA reader. Cut-off values were determined by calculating the mean and the standard deviation of the OD of four determinations from the negative control serum pool.[8] A serum sample was considered positive when its OD was greater than the control mean plus two standard deviations. The same control pool was used in all determinations.[8]

Antigen detection by ELISA (AG-ELISA)

Cerebrospinal fluid was diluted 1:160 in bicarbonate buffer, and 100 μl was placed in duplicate wells of microtiter plates (Immulon 1). The plates were incubated overnight at 4°C and washed five times with PBS-Tween. Rabbit antisera to cysticercosis (100 μl) at a 1:5,000 dilution in PBS-Tween was incubated in the wells for 1 hr at 37°C. The plates were then washed five times with PBS-Tween and 100 μl of peroxidase-conjugated goat anti-rabbit IgG (1:1,000 dilution) was added. The plates were incubated for 1 hr at 37°C, washed five times with PBS-Tween, and 100-μl volumes of freshly prepared substrate (0.4 mg of *o*-phenylenediamine/ml plus 0.06% H_2O_2 in citrate-phosphate buffer, pH 5) were added. The reaction was allowed to proceed for

TABLE 1

*Sensitivity of the enzyme-linked immunoelectrotransfer blot (EITB) and the enzyme-linked immunosorbent assay (ELISA) with serum and cerebrospinal fluid (CSF) of Peruvian patients with confirmed cysticercosis**

Patient description (clinical groups)	EITB No. positive/no. tested (%)		AB-ELISA No. positive/no. tested (%)		AG-ELISA† No. positive/no. tested (%)
	Serum	CSF	Serum	CSF	CSF
Subcutaneous cysts	12/14 (88)	7/8 (89)	12/15 (80)	6/8 (78)	2/5 (40)
Ocular cysts	3/3 (100)	1/1 (100)	2/3 (67)	0/1 (0)	0/1 (0)
Subcutaneous and ocular cysts	2/2 (100)	1/1 (100)	2/2 (100)	1/1 (100)	0/0 NA
Cysts at other locations	15/15 (100)	9/11 (82)	6/14 (43)	6/11 (55)	8/9 (89)
All confirmed cases	32/34 (94)‡	18/21 (86)§	22/34 (65)	13/21 (62)	10/15 (67)

* AB-ELISA = antibody ELISA; AG-ELISA = antigen ELISA; NA = not available.
† Only CSF can be used in the AG-ELISA.
‡ $P < 0.05$ versus AB-ELISA for serum, by chi-square test.
§ $P > 0.05$ versus AG-ELISA for CSF, by chi-square test.

5 min at room temperature, and was stopped by the addition of 25 μl of 1 M sulfuric acid. The OD was measured at 492 nm in a photometer. A sample was considered positive when its OD was greater than the mean of the negative control plus two standard deviations.

Antibody detection by immunoblot (EITB)

Glycoprotein antigens were prepared as previously described.[7] Cysts were dissected and washed as described above, drained, and blotted dry. They were suspended in 20 ml (two times the weight of the cysts) of 8 M urea in Tris HCl buffer (0.05 M Tris HCl, 0.1 M NaCl, pH 8.0), homogenized, and sonicated as described above. Fifty percent freon was added, and the material was centrifuged for 30 min at 15,000 rpm (SA-600 Rotor) at 4°C. The supernatant was collected, centrifuged at 250,000 *g* for 2 hr, desalted with Sephadex G-25, and passed through a lentil-lectin Sepharose 4B column at room temperature. Lentil-lectin–bound glycoproteins were eluted from the column with 0.2 M α-methyl-mannoside in Tris HCl buffer. After the protein concentration was determined, the glycoprotein antigens were stored in glycerol.

Lentil-lectin–purified glycoprotein antigens were used in an immunoblot format to detect infection-specific antibodies in serum and CSF, as previously described.[7] Peroxidase-labeled goat anti-human antibody was used at a 1:1,000 dilution. Bound antibodies were visualized using the H_2O_2/DAB substrate system.[7] Seven glycoprotein bands are commonly recognized by antibodies from serum of cysticercosis patients.[7] These glycoprotein bands have molecular masses of 50, 42–39, 24, 21, 18, 14, and 13 kD, respectively. Quality control analysis of the EITB assay was performed in collaboration with the PDB laboratory at CDC.

TABLE 2

Specificity of the enzyme-linked immunoelectrotransfer blot (EITB) and the antibody enzyme-linked immunosorbent assay (AB-ELISA) for antibody to Taenia solium *in serum samples of negative controls from Bangladesh and the United States*

Serum source (negative controls)	EITB No. negative/no. tested (%)	AB-ELISA* No. negative/no. tested (%)
Bangladesh	51/51 (100)	32/51 (63)
United States	11/11 (100)	10/11 (91)

* The AB-ELISA is significantly more specific ($P < 0.05$ by chi-square test) with serum samples from the United States than with those from Bangladesh.

Data analysis

The sensitivity and specificity of the ELISA and EITB with sera and CSF were calculated. Only confirmed cysticercosis cases and negative controls were used in the calculation of specificity and sensitivity. The chi-square test was used for

TABLE 3

Sensitivity and specificity of the enzyme-linked immunoelectrotransfer blot (EITB) and the enzyme-linked immunosorbent assay (ELISA) for antibody to Taenia solium *in serum and cerebrospinal fluid (CSF) of patients**

Parasitologically confirmed disease†	EITB No. positive/no. tested (%)		AB-ELISA No. positive/no. tested (%)		AG-ELISA‡ No. positive/no. tested (%)
	Serum	CSF	Serum	CSF	CSF
T. solium with neurologic symptoms	4/4 (100)	3/3 (100)	4/4 (100)	2/3 (67)	1/3 (33)
T. solium without neurologic symptoms	10/10 (100)	1/1 (100)	4/6 (67)	0/1 (0)	0/1 (0)
Total *T. solium*	14/14 (100)	4/4 (100)	8/10 (80)	2/4 (50)	1/4 (25)
T. saginata	0/4 (0)	0/1 (0)	2/4 (50)	0/1 (0)	0/1 (0)
Echinococcus granulosus	0/18 (0)	—	2/18 (11)	—	—
Hymenolepes nana	1/59 (2)	—	12/59 (20)	—	—
Diphyllobothrium sp.	0/2 (0)	—	0/2 (0)	—	—

* AB-ELISA = antibody ELISA; AG-ELISA = antigen ELISA.
† All patients either passed worms or had tissue-confirmed disease.
‡ Only CSF can be used in the AG-ELISA.

comparison of data sets. *P* values less than 0.05 were considered significant.[9]

RESULTS

Sensitivity and specificity

The EITB was the most sensitive test with either sera or CSF specimens (Table 1). The sensitivity with CSF was similar for both the AB-ELISA and AG-ELISA tests. Surgery patients with hydrocephalus tended to have more false-negative results in the AB-ELISA assay than did patients with other clinical presentations (Table 1).

The EITB assay was also highly specific (Table 2). In contrast, the AB-ELISA gave relatively nonspecific results, especially with Bangladeshi serum samples. With the serum samples from the United States, the AB-ELISA showed a higher specificity (Table 2), but its specificity was still not comparable to that of the EITB. The specificity of the AG-ELISA, which requires CSF, was not studied because CSF samples from heterologous infections were not available.

Cross-reactivity

Eighteen Peruvian patients with tissue-confirmed *E. granulosus* (hydatid) infections and 59 Peruvian patients with *H. nana* infections were tested (Table 3). Two (11%) hydatid and 12 (20%) *H. nana*-positive serum samples reacted in the AB-ELISA. In contrast, when these sera were tested by EITB, none of the patients with hydatids and only 1 with a *H. nana* infection had a positive result, which consisted of three bands specific for cysticercosis (GP50, GP42-39, and GP24). Because these 18 patients reside in an area where cysticercosis is endemic, the one with *H. nana* may also be infected with cysticercosis.

Patients with intestinal taeniasis

All serum samples from the four patients with neurologic symptoms who had passed proglottid segments identified as *T. solium* were positive by both AB-ELISA and EITB (Table 3). Ten persons with *T. solium* worms were neurologically asymptomatic. Serum samples from these patients were all positive by EITB, but only six (60%) were positive by AB-ELISA. Serum samples from four patients who had passed *T. saginata* proglottids were negative by the EITB test, but two reacted positively on the AB-ELISA test. Neither of the serum samples from the two patients who had passed *Diphyllobothrium* sp. was positive by EITB or AB-ELISA (Table 3).

Reproducibility

Twenty-one serum and CSF samples were sent to CDC for analysis by EITB. All positive samples were also positive at CDC (100% concordance). Concordance of the bands in confirmed patients was 91%. All sera were retested by AB-ELISA by an independent observer. No significant difference was observed in the two sets of results.

Test costs

We estimated the cost per 100 tests to be $53 for the EITB and $15 for the two ELISAs. We excluded the cost of labor, shipping, depreciation, and equipment. The EITB test uses over ten times more conjugate than does the ELISA. This difference represents the major cost difference between the two tests. Other significant costs in the EITB test are the nitrocellulose paper and the purified antigen. The power source and chambers for the EITB cost about the same as the cheapest ELISA reader on the market.

DISCUSSION

All reagents for each assay were individually assayed for both sensitivity and specificity. Our results demonstrate that the EITB is the best available test for the diagnosis of cysticercosis infection. The test has a high sensitivity and specificity for antibody to *T. solium* in both serum or CSF specimens. Since the EITB detects antibody with equal efficacy in both serum and CSF, it can be used with nonhospitalized patients, and is highly applicable for epidemiologic studies.

Neither the AB-ELISA, using a crude *T. solium* antigen, nor the AG-ELISA was nearly as sensitive as the EITB test. In addition, the AB-ELISA was not very specific. Previous studies on porcine cysticercosis in Peru also demonstrated that the EITB is a more sensitive and specific test than the ELISA.[10] Several previous studies have reported a high sensitivity for the AG-ELISA. However, these studies may be biased because they selected as their positive controls cysticercosis patients who had had antibody detected by another test.[6, 11]

Cross-reactive components in antigen preparations are the most important obstacles to overcome in producing a reliable test. The current ELISA uses a crude or slightly purified antigen that shows cross-reactivity. This phenomenon occurs even with the lentil-lectin–purified antigens used for the EITB assay; however, in this test, irrelevant bands are easily distinguished from those of the specific antigens by the differences in their molecular mass. In the ELISA, specific reactions cannot be differentiated from cross-reactions.

Sera from patients infected with *H. nana,* a common helminthic infection, frequently cross-react in the AB-ELISA.[12] In areas where this parasite is endemic, high rates of false-positive results will occur. In contrast, the EITB test for cysticercosis does not show cross-reactivity with *H. nana* or any other helminthic infections in patients from areas without endemic cysticercosis. The single patient with an *H. nana* infection whose serum reacted in the EITB was a resident of Peru, an area where cysticercosis is endemic. Although the patient has no direct evidence of this disease, we cannot rule out an occult coinfection by *T. solium* in this individual.

The selection of populations to study test specificity is a critical issue in tropical diseases. Our selection of the Bangladeshi negative controls, who are also from a tropical developing country and are similar to our Peruvian patients in terms of socioeconomic background but do not have any risk for cysticercosis, is an attempt to achieve the ideal negative control. Since the development of the EITB for cysticercosis, over 12,000 assays have been performed, and not a single false-positive case has been documented. The 100% specificity of this assay can be tested properly only with carefully selected negative controls from the developing world.

Our laboratory in Peru can perform 300–500 EITB tests per week. If conjugates are prepared in-house, the cost of the EITB is similar to that of ELISA. The EITB assay is the best current immunodiagnostic test for cysticercosis and/or taeniasis. The next task is to develop inexpensive immunologic tests that can be performed in the field or in minimally equipped laboratories, while retaining the high sensitivity and specificity of the EITB.

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REFERENCES

1. Mahajan RC, 1982. Geographical distribution of human cysticercosis. Flisser A, Willms K, Laclette JP, Larralde C, Ridaura C, Beltran F, eds. *Cysticercosis: Present State of Knowledge and Perspectives.* New York: Academic Press, 39–46.
2. Schenone H, Ramirez R, Rojas A, 1973. Aspectos epidemiologicos de la neurocisticercosis en America Latina. *Bol Chil Parasitol 28:* 61–72.
3. Schenone H, Villarroel F, Rojas A, Ramirez R, 1982. Epidemiology of human cysticercosis in Latin America. Flisser A, Willms K, Laclette JP, Larralde C, Ridaura C, Beltran F, eds. *Cysticercosis: Present State of Knowledge and Perspectives.* New York: Academic Press, 25–38.
4. Nash TE, Neva FA, 1984. Recent advances in the diagnosis and treatment of cerebral cysticercosis. *N Engl J Med 311:* 1492–1496.
5. Diwan A, Coker-Vann M, Brown P, Subianto DB, Yolken R, Desowitz R, Escobar A, Gibbs Jr C, Gajdusek C, 1982. Enzyme-linked immunosorbent assay (ELISA) for the detection of antibody to cisticerci of Taenia solium. *Am J Trop Med Hyg 31:* 364–369.
6. Estrada J, Kuhn R, 1985. Immunochemical detection of antigen of larval Taenia solium and anti-larval antibodies in the cerebrospinal fluid of patients with neurocysticercosis. *J Neurol Sci 71:* 39–48.
7. Tsang VCW, Brand J, Boyer E, 1989. Enzyme-linked immunoelectrotransferency blot assay and glycoprotein antigens for diagnosing human cysticercosis (Taenia solium). *J Infect Dis 159:* 50–59.
8. Larralde C, Laclette JP, Owen CS, Madrazo I, Sandoval M, Bojalil R, Sciutto E, Contreras L, Arzate J, Diaz ML, Govezensky T, Montoya RM, Goodsaid F, 1986. Reliable serology of Taenia solium cysticercosis with antigens from cyst vesicular fluid: ELISA and hemagglutination tests. *Am J Trop Med Hyg 35:* 965–973.
9. Armitage P, Berry G, 1987. *Statistical Methods in Medical Research.* London: Blackwell Scientific Publications, 472.
10. Gonzales AE, Cama V, Gilman RH, Tsang VCW, Pilcher JB, Chavera A, Castro M, Montenego T, Verastegui M, Miranda E, Bazala H, 1990. Prevalence and comparison of serologic assays, necropsy, and tongue examination for the diagnosis of porcine cysticercosis in Peru. *Am J Trop Med Hyg 43:* 194–199.
11. Tellez-Giron E, Ramos MC, Dufour L, Alvarez P, Montante M, 1987. Detection of Cysticercus cellulosae in cerebrospinal fluid by dot enzymne-linked immunosorbent assay (DOT-ELISA) and standard ELISA. *Am J Trop Med Hyg 37:* 169–173.
12. Castillo R, Grados P, Carcamo C, Guevara A, Verastegui M, Gilman RH, 1991. Effect of treatment on serum antibody to Hymenolepis nana detected by enzyme-linked immunosorbent assay. *J Clin Microbiol 29:* 413–414.