Enzyme-Linked Immunosorbent Assay for the Diagnosis of Cerebral Cysticercosis

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Central nervous system cysticercosis is common in countries where Taenia solium occurs in pigs and the level of hygiene and sanitation is low. The clinical presentation may include epileptic seizures, focal neurological deficits, hydrocephalus, or aseptic meningitis. The disease is frequently seen in California residents of Hispanic origin. It sometimes occurs in whites from homes that employ Hispanic cooks. Diagnosis is often difficult. Computerized tomography scan and brain biopsy are the most reliable diagnostic procedures, but each has its limitations. We have found that a radioimmunoassay improves our diagnostic capability, and more recently we have adapted this to an enzyme-linked immunosorbent assay that is equally sensitive and specific and, in addition, obviates the need for radioactive materials. Details of the enzyme-linked immunosorbent assay procedure and its application to the diagnosis of central nervous system cysticercosis form the basis of this report.

Cysticercosis is a frequent cause of neurological disease where the pork tapeworm, Taenia solium, occurs in humans and the larval stage exists in pigs. In Mexico and in bordering areas of the United States, cerebral cysticercosis is often seen in neurology clinics (8). It was found in 1.9% of autopsies in Mexico (4), and 1% of the population had positive serology in epidemiological surveys (3). At the Harbor-University of California, Los Angeles, Medical Center, about 20 new cases are seen yearly, and the disease involves both adults and children.

Cerebral cysticercosis is usually acquired by ingestion of food or water that harbors ova of the pork tapeworm, usually as a result of fecal contamination. The adult worm, like its beef counterpart (Taenia saginata), inhabits the human intestine, where it produces few or no symptoms. The larval stage, which normally occurs in pigs, may develop in humans as a result of ova gaining access to the stomach. In the stomach, the shells of the ova break open, and the larvae, liberated from the eggs, penetrate the intestinal mucosa and are carried to many parts of the body, notably subcutaneous tissues, muscles, eyes, and brain, where they develop and form cystic structures called cysticerci. In these locations, the cysticerci live for many years; after their death, the cysts may calcify. Cysts in the subcutaneous tissue and muscle seldom produce significant symptoms, but in the brain variable symptoms may develop, depending on the location of the cyst(s). Cortical and subcortical cysts often mimic brain tumors or give rise to seizures (12, 15). Ventricular cysts may cause symptoms as a result of disturbances in the circulation of cerebrospinal fluid (CSF), leading to increased intracranial pressure and hydrocephalus (2, 7, 15). Meningal cysts may give rise to aseptic meningitis (15).

The diagnosis of cerebral cysticercosis is often difficult, but the following tests have been frequently used: indirect hemagglutination (6), complement fixation (6), brain biopsy, and computerized tomography scan (12, 14).

To improve the serological diagnosis of this disease, we developed a radioimmunoassay (RIA) for the determination of specific immunoglobulin G (IgG) antibodies to Cysticercus cellulosae (10). In this study, we report the adaptation of the RIA procedure to an enzyme-linked immunosorbent assay (ELISA) for the diagnosis of cysticercosis. The ELISA has been used in the serological diagnosis of other parasitic diseases such as malaria, schistosomiasis, trypomani- somiasis, and leishmaniasis (19). The technique is simple, inexpensive, reproducible, and does not expose personnel to radioactive materials. A preliminary report has been presented (I. N. Mohammad et al., Clin. Res. 31:129A, 1983).

MATERIALS AND METHODS

Antigen preparation. To 100 mg of C. cellulosae teased from porcine muscle and lyophilized was added 15 ml of cold anhydrous ether followed by grinding for 10 to 15 min in a tissue grinder immersed in an ice bath. The suspension was centrifuged in the cold for 30 min at 850 × g. After removal of supernatant, the delipidized sediment was suspended in 15 ml of Veronal bicarbonate-buffered saline and ground for an additional 10 min at room temperature. This was followed by agitation at 4°C for 4 h and centrifugation at 850 × g in a refrigerated centrifuge. Samples (1 ml) of opalescent supernatant were then transferred to glass ampules, freeze-dried, and stored at −20°C until used. Veronal bicarbonate-buffered salt was prepared by dissolving the following in 1 liter of distilled water: 8.4 g of NaCl, 0.25 g of NaHCO₃, 0.3 g of sodium 5,5-diethylbarbiturate, and 0.46 g of 5,5-diethyl barbituric acid. The final pH was 7.35.

A commercial antigen obtained from George S. Tullock and Associates, San Antonio, Tex., has also been utilized and proved to be adequate in differentiating most positive sera and CSF from normal sera and CSF. Borderline sera and CSF antibody values, however, were better separated from normal values by the antigen prepared at the Communicable Disease Center, Atlanta, Ga., as described above.

Serum and CSF specimens. Nineteen paired serum and CSF specimens from patients with cerebral cysticercosis confirmed by diagnostic computerized tomography scan or brain biopsy or both were studied. Sera and CSF from 19
Hispanic patients with other neurological diseases served as controls. A serum pool obtained from 12 healthy white rabbits of the same sex and age and CSF from agammaglobulemic patients were also included in the assay. All specimens had been previously studied by RIA.

Preparation of antiserum. Human immunoglobulin, Cohn fraction 2, was further purified by DEAE-Sephadex chromatography and was shown by immunoephoresis and the Ouchterlony technique to be at least 99% IgG. This purified IgG was used to immunize New Zealand white rabbits. Purified IgG (4 mg) in 0.4 ml of normal saline was emulsified in 0.6 ml of complete Freund adjuvant, and 0.25 ml was injected subcutaneously behind each shoulder and 0.5 ml was injected intraperitoneally into each of six rabbits. Each rabbit received three injections at 1-month intervals. Two weeks after the final immunization and weekly thereafter for 4 weeks, the animals were bled from ear veins. The sera obtained were tested and pooled, and the pool was repeatedly absorbed with insolubilized IgA, IgE, and IgD myeloma proteins and insolubilized IgM isolated from a macroglobulinemic serum. The absorbed antiserum was shown by immunoelectrophoresis, Ouchterlony analyses, and RIA to be specific for IgG.

Conjugate. Goat anti-rabbit IgG alkaline phosphatase conjugate and the enzyme substrate, p-nitrophenyl phosphate, were purchased from Sigma Chemical Co., St. Louis, Mo.

ELISA procedure. Dynatech Immulon plates with removable wells were used in this study. The plates and reagents were evaluated, and optimal conditions for the assay were determined in preliminary tests. A modified double-antibody sandwich method was employed (18, 19). The essentials of the procedure were as follows. The cysticercus antigen was solubilized in 0.01 M phosphate-buffered saline (pH 7.2) at an approximate concentration of 0.1 mg/ml (optical density [OD] at 280 nm, 0.11). Antigen solution (0.1 ml) was added to each well, and the plate was incubated for 2 h at 37°C. At the end of the incubation, the antigen solution was removed, and the reservoirs were washed three times with phosphate-buffered saline containing 0.05% Tween 20. Serum (0.1 ml) at a dilution of 1:1.000 or CSF diluted 1:20 in phosphate-buffered saline was next added to each well. Again the plate was incubated for 1 h, followed by washing with phosphate-buffered saline–0.05% Tween 20 as above. Rabbit antihuman IgG (0.1 ml) diluted 1:1,000 was then added to each well, followed by incubation and washing. Goat anti-rabbit IgG alkaline phosphatase conjugate (0.1 ml) diluted 1:2,000 was added to each well. The plate was incubated for 1 h and washed, and 0.1 ml of enzyme substrate solution (p-nitrophenyl phosphate, 1 mg/ml in diethanolamine buffer, pH 9.8) was added. The plate was wrapped in aluminum foil to exclude light and incubated for 1 h at room temperature, after which the OD was read in an automated spectrophotometer at a wavelength of 405 nm.

Statistical analysis. The mean and standard deviations of the mean for the optical density (ELISA) and percent binding (RIA) were calculated. Logarithmic transformation of the data was accomplished to correct for differences in the standard deviations of the mean values, and the results were analyzed by the paired sample t-test. P values less than 0.05 were considered significant.

To evaluate the reliability of ELISA for the diagnosis of central nervous system (CNS) cystercerosis, sensitivity, specificity, positive and negative predictive values were calculated for tests on serum and CSF. For the purposes of these calculations, the prevalence of cerebral cystercerosis in patients admitted to this hospital was estimated to be 0.5%. Approximately 50% of admitted patients at the present time are Mexican-American. Since 1.9% of this population has cerebral cystercerosis at postmortem (4), a somewhat lesser percentage of the general population could be expected to have the disease. Since 1% of Mexicans have been reported to have positive serology (3), we assume that this is a reasonable approximation of the prevalence of cystercerosis in Mexican-Americans coming to this hospital. Some will have noncerebral cystercerosis, which suggests less than 1% would have the cerebral form of the disease.

Sensitivity, specificity, and predictive values were calculated according to the following formulas: percent sensitivity = [(true-positive)/(true-positive + false-negative)] × 100; percent specificity = [(true-negative)/(true-negative + false-positive)] × 100; predictive value positive = [(sensitivity × prevalence)/(sensitivity × prevalence) + (1 – specificity)(1 – prevalence)] × 100; and predictive value negative = [(specificity (1 – prevalence))][specificity (1 – prevalence)] × 100.

RESULTS

Table 1 is a comparison of ELISA and RIA studies done on identical serum samples. The mean OD of tests on the patients’ sera was 1.33, whereas for the control sera it was 0.34. The difference between these two groups was highly significant (P < 0.005). The ODs for pooled normal serum and for serum from an agammaglobulinemic patient are included for comparison. By RIA, the mean percent binding for the patients’ sera was 58.58, whereas that of the control sera was 33.58. The difference is also highly significant (P < 0.005).

Figure 1 shows the results in a scattergram. Most of the control values fell on the left side of the figure, whereas the patient values are seen to the right, with a slight overlap. The coefficient of correlation (r) between the two techniques is 0.96 (P < 0.001). One patient’s value fell within the control group values. This patient had a calcified cyst in the brain, which on subsequent followup by computed tomography scan was found to have disappeared.

Table 2 shows the results of studies on CSF. Patients’ CSF showed a mean OD of 1.44, whereas the controls’ CSF had a mean OD of 0.28. Again the difference between these groups was significant (P < 0.005). The results of the RIA were similar. A comparison of the two techniques is presented in Fig. 2. Again most of the control values lie to the left of the graph (r = 0.65), whereas nearly all of the patients’ values lie to the right (r = 0.87). There is little overlap. The coefficient of correlation of the two groups combined is 0.94 (P < 0.001). The data from the patient described above again overlapped with those of the controls. Also, the data from one control overlapped with those of the patients. This patient had Guillain-Barre syndrome. The possibility that he

<table>
<thead>
<tr>
<th>Specimen</th>
<th>ELISA (OD)</th>
<th>RIA (% binding)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pooled normal serum</td>
<td>0.34</td>
<td>38.88</td>
</tr>
<tr>
<td>Agammaglobulinemic serum</td>
<td>0.16</td>
<td>0.54</td>
</tr>
<tr>
<td>Patient sera (n = 19)</td>
<td>1.33 ± 0.33</td>
<td>58.58 ± 10.49</td>
</tr>
<tr>
<td>Control sera (n = 19)</td>
<td>0.34 ± 0.09</td>
<td>33.58 ± 5.12*</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation (range within parentheses).
* t = 15.19, P < 0.005.
* t = 9.76, P < 0.005.
may have had concurrent or prior inapparent cysticercosis could not be ruled out.

Table 3 shows the results of studies done on the sera and CSF from patients according to a clinical classification of the cysticercosis. Patients with ventricular cysts and hydrocephalus all had detectable antibodies to the cysticercus antigen in both serum and CSF. Patients who had meningitis all had antibodies in the serum, and all except one had antibodies in the serum. Only two of three patients with parenchymal cysts had detectable antibodies in serum, and two had CSF antibodies. All three had increased antibodies in either serum or CSF. The one patient alluded to above had a negative test for IgG antibodies in the serum, whereas a second patient with a persistent calcified cyst was negative for CSF antibodies. The third patient had parenchymal cysts that had not yet calcified. His serum and CSF had high levels of antibody.

The ELISA test on the serum has a sensitivity of 89.5% and a specificity of 100%. Similarly, the sensitivity and specificity of the CSF test were each 94.7%. Thus, the sensitivity of the CSF test appears to be slightly greater than that of the serum test, whereas the serum test shows slightly greater specificity. We believe it is often useful to study both serum and CSF in considering the diagnosis of cerebral cysticercosis.

**TABLE 2.** Comparison of ELISA and RIA studies of CSF in the diagnosis of cerebral cysticercosis

<table>
<thead>
<tr>
<th>Specimen</th>
<th>ELISA (OD)</th>
<th>RIA (% binding)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal CSF</td>
<td>0.11</td>
<td>0.01</td>
</tr>
<tr>
<td>Agammaglobulinemic CSF</td>
<td>0.14</td>
<td>0.08</td>
</tr>
<tr>
<td>Patient CSF (n = 19)</td>
<td>1.44 ± 0.30*</td>
<td>48.85 ± 20.53</td>
</tr>
<tr>
<td></td>
<td>(0.36-1.67)</td>
<td>(2.36-73.87)</td>
</tr>
<tr>
<td>Control CSF (n = 19)</td>
<td>0.28 ± 0.19*</td>
<td>2.70 ± 1.46</td>
</tr>
<tr>
<td></td>
<td>(0.14-1.00)</td>
<td>(1.47-6.87)</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation (range within parentheses).

**FIG. 1.** Comparison of serum antibodies to *C. cellulosae* measured by ELISA and RIA. The figure shows a good correlation between the two techniques, \( r = 0.96 \). An OD of 0.70 (i.e., greater than or equal to twice the mean value of all negative controls) was considered abnormal.

**FIG. 2.** Comparison of CSF antibodies to *C. cellulosae* measured by ELISA and RIA. The coefficients of correlation were as follows: for the two groups combined, \( r = 0.94 \); for the control group, \( r = 0.65 \); for the patients, \( r = 0.87 \). An OD of 0.6 (i.e., greater than or equal to twice the mean value of all negative controls) was considered abnormal.

Calculated positive and negative predictive values are shown in Table 4. For purposes of these calculations, the specificity of the serum test was taken to be 92.7%, which represents the center of 95% confidence interval based on a primordial distribution. To determine true predictive values, the prevalence of cysticercosis in the population under study was employed (see formulas, above). As alluded to above, the prevalence of cysticercosis among the Hispanic population in southern California is estimated to be 0.5 to 1.0%. However, experience to date suggests that the highly selected neurologically normal patients whose sera are submitted for serological study in this hospital may comprise a group with a prevalence of cerebral cysticercosis as high as 50%. Therefore, in Table 4 predictive values for the test are given for a range of prevalence rates. Positive predictive values increase for tests on both serum and CSF as the prevalence rate rises. If one were studying all Hispanic patients admitted to this hospital, the prevalence rate would lie between 0.5 and 25. For all Hispanic subjects with neurological disease, the prevalence may approach 25%, and when other readily diagnosed neurological disorders have been ruled out and cysticercosis is a prime suspect, the prevalence approaches 50%. During the past 25 years, the sera of 121 patients with suspected cases of CNS cysticercosis have been submitted for analysis, and 43% have had a positive test on serum or CSF (39% had a positive serum test, and 39% had a positive test on the CSF).

**TABLE 3.** Results of ELISA test on serum and CSF antibodies of patients with different clinical forms of cerebral cysticercosis

<table>
<thead>
<tr>
<th>Clinical presentation</th>
<th>No. of cases</th>
<th>Positive serum</th>
<th>Positive CSF</th>
<th>Positive serum or CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meningitis</td>
<td>8</td>
<td>7</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Ventricular cysts</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Parenchymal cysts</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Hydrocephalus</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

\( \text{Mean} \pm \text{standard deviation (range within parentheses).} \)

\( t = 12.54, P < 0.005. \)

\( t = 12.98, P < 0.005. \)
TABLE 4. Predictive values calculated for tests on serum and CSF at a given prevalence rate of cysticercosis

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>Serum Positive</th>
<th>Serum Negative</th>
<th>CSF Positive</th>
<th>CSF Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>5.8</td>
<td>99.9</td>
<td>8.3</td>
<td>99.9</td>
</tr>
<tr>
<td>1.0</td>
<td>11.0</td>
<td>99.8</td>
<td>15.4</td>
<td>99.9</td>
</tr>
<tr>
<td>25.0</td>
<td>80.3</td>
<td>96.3</td>
<td>85.7</td>
<td>98.2</td>
</tr>
<tr>
<td>50.0</td>
<td>92.5</td>
<td>89.8</td>
<td>94.7</td>
<td>94.7</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The clinical presentation of cerebral cysticercosis is highly variable. Brain tumor is frequently suspected (17). Ventricular cysts are potentially life threatening as evidenced by two sudden deaths observed in our hospital due to ventricular cysticerci.

The diagnosis of CNS cysticercosis is often difficult. In endemic areas, the disease is a prime suspect in patients with findings suggesting a brain tumor. Palpation for subcutaneous nodules and roentgenograms for calcified cysts in muscles occasionally aid the diagnosis (16). In the past, indirect hemagglutination and complement fixation tests have been utilized, but in our experience, neither has been sufficiently reliable to permit the desired degree of confidence in serological diagnosis. Recently, computerized tomography scans have been employed with considerable success and are especially helpful in determining the need for surgery (12, 14).

Because concern has been expressed by others over serological cross-reactions in patients with echinococcosis (1, 13), we studied the sera of 20 patients with proven echinococcosis who had positive indirect hemagglutination tests (sera provided by the Centers for Disease Control, Atlanta). Eight of these showed markedly elevated antibodies to crude cysticercal antigen, six had slightly increased antibodies, and six had values within the normal range for the controls. The possibility that some of these patients might have had concurrent or previous cysticercosis could not be ruled out. However, since hydatid disease is rarely a problem in the Hispanic population we service (20), and since cerebral echinococcal disease is an extreme rarity in our hospital, we have found this assay to be highly specific and very helpful in making the diagnosis of CNS cysticercosis in the population we service. In localities where hydatid disease is prevalent, the immunological diagnosis of cysticercosis must be made with due caution. The use of whole worm (T. solium) antigen has been reported to be helpful in reducing serological cross-reactivity in patients with echinococcosis (1). Further work is warranted in developing parasite-specific immunosassays for these two conditions.

Our current study extends the work of Arambulo et al. (1), who pointed out the potential value of ELISA in the serodiagnosis of cerebral cysticercosis. We have found the study of both serum and CSF to be helpful in the evaluation of patients in whom cerebral cysticercosis is suspected. In our experience, most subjects in whom the diagnosis was proved have had abnormal antibodies in both serum and CSF. However, a few patients with calcified parenchymal cysts have had antibodies only in serum or in CSF. Also, when a borderline value is found on testing the serum, assay of the CSF may provide the correct answer. Antibodies were not found in either the serum or CSF of 19 Hispanic control subjects with known noncysticercal CNS disease or in 15 white control subjects from whom paired serum and CSF were available for study. Thus, in our hands the ELISA procedure proved to be a significant improvement in the diagnosis of CNS cysticercosis. Others have reported 20 to 40% false-negative and false-positive results with either indirect hemagglutination (9) or complement fixation tests (9, 11), a notable problem being false-negative results in patients with ventricular or parenchymal cysts.

Preliminary studies in our laboratory indicate that with appropriate specific antisera, ELISA can be used to measure class-specific as well as subclass-specific antibodies to antigens of cysticerci. Other investigators have shown IgG antibodies to be the dominant antibody class in patients with cysticercosis. IgM, IgE, and IgA antibody classes have been detected to a lesser extent (5). Our findings are in agreement with this and suggest that IgD antibodies as well as IgG subclass antibodies are also measurable in many patients with RIA or ELISA techniques.

We conclude that elevated levels of IgG antibodies to C. cellulosae in serum, CSF, or both in subjects suspected of having cysticercosis strongly suggests cysticercosis. Antibody values within the normal range in both serum and CSF indicate that cysticercosis is highly unlikely. Finally, the ELISA technique has similar sensitivity and specificity to RIA, yet is simpler and less expensive to perform. Hence, the ELISA promises to be very useful in the diagnosis of cerebral cysticercosis. The ELISA should prove to be invaluable in areas where the disease is common and facilities or personnel are inadequate to perform RIAs.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


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ELISA FOR THE DIAGNOSIS OF CEREBRAL CYSTICERCOSIS


