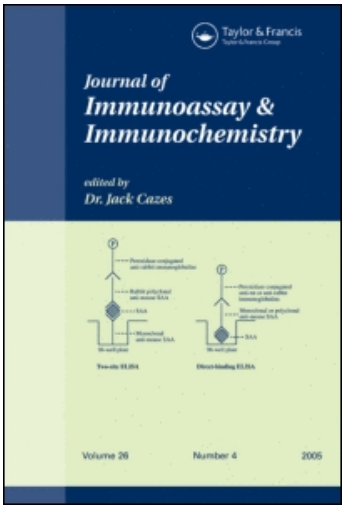

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A Micro Elisa for the Diagnosis of Cerebral Cisticercosis

R. Nunez[a]; A. Munoz[a]; C. Nunez[a]; B. Gomez[a]
[a] Facultad de Medicina Pediatric Department, Immunology Division Hospital Infantil, Servicio de Salud de Caldas Cedri, Universidad de Caldas, Caldas, Colombia

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A MICRO ELISA FOR THE DIAGNOSIS OF CEREBRAL CISTICERCOSIS

R Nunez, A Munoz, C Nunez, and B Gomez
Universidad de Caldas, Facultad de Medicina
Pediatric Department, Immunology Division
Hospital Infantil, Servicio de Salud de Caldas
Cedri, Manizales, Caldas, Colombia

ABSTRACT

A micro ELISA for cysticercosis in the central nervous system was evaluated in 24 patients and compared with indirect hemagglutination. CSF ELISA results were positive in 17 of 20 patients (sensitivity 85%), whereas CSF indirect hemagglutination was positive in only 14 patients (sensitivity 70%).

KEY WORDS: Cerebral Cysticercous, Micro ELISA, Serum, CSF, Sensitivity.

INTRODUCTION

Cysts of Taenia solium have been found in the nervous system (cerebral cysticercosis), eyes, striate muscles, heart and subcutaneous tissues. Cysts of Taenia solium in the brain produce seizures, increased intracranial pressure, mental changes, headache, gait difficulties, cranial nerve palsies, visual disturbances, and death.

Cerebral cysticercosis has been considered a rare disease in Colombia, with neurosurgical and necropsy findings being the only means for making a diagnosis. Until 1983 less than 200 cases had been found. In

1964 Lopez and Escandon (1) described 58 patients with cerebral cysticercosis in post-mortem studies performed in Medellin, Colombia. In post-mortem studies at San Vicente de Paul Hospital, cerebral cysticercosis was detected in 0.7% of patients coming to necropsy.

With the development of immunological techniques and CT scanning, the prevalence of diagnosis of cerebral cysticercosis has recently increased in Colombia (2,3,4). In a previous study involving 77 patients with clinical signs suggestive of cerebral cysticercosis, 24 were confirmed by CT scan, biopsy and antibodies. Two of these patients were infants. We have also studied 322 healthy blood bank donors, and in 24 (7.5%) antibodies were detected against cisticercous antigens by the indirect hemagglutination test. The high prevalence of antibodies might be due to benign infection or cross-reaction with other parasites (5). Indirect hemagglutination is inexpensive and sensitive but it has technical limitations, and for this reason the ELISA, a more sensitive and specific test has also been used for cysticercosis (6).

Ramirez (9) recently presented a study of ELISA in patients with established diagnoses of cerebral cysticercosis using both serum and CSF. They found 92% of patients had serum antibodies while only 31% had CSF antibodies. We describe here a new ELISA for cysticercosis and compare it with indirect hemagglutination in both serum and CSF.

MATERIAL AND METHODS

Twenty-four patients with cerebral cysticercosis were diagnosed by CT scan, skull X-rays, and biopsy in 10 patients. CSF from 9 patients

without cerebral cysticercosis but with seizures (5), cerebral tumors (2) and infectious diseases of CNS (2) were studied; as well as sera from 10 healthy laboratory workers.

Immunological tests

a. Indirect hemagglutination (IH): Sheep red blood cells were sensitized with formaldehyde 10% and Taenia solium antigen (0.1 g/L), obtained from the National Institute of Health of Colombia. A titer greater than or equal to 1 in 16 was considered positive.

b. ELISA test for anticycticercal antibodies in serum and CSF: The technique was as follows: 5 μL of Taenia solium antigen (0.1 g/L) in 5 μL carbonate buffer, 100 mmol/L, pH 9.6, was added to polystyrene tubes, at $4^{o}C$ for 12 hours, then washed 5 times with phosphate buffered saline, pH 7.6, with 0.01% Tween 20. To each tube was added 5 μL of serum diluted 1:10 in PBS-Tween 20, or CSF without dilution. After 30 minutes at room temperature, and 4 washes, anti-IgG-peroxidase (5 μL) was added to each tube and incubated for 30 minutes. After 4 washes, 200 μL of orthophenylendiamine (OPD), 2.56 mg/cc plus 0.02% H_2O_2 in citrate buffer was added and incubated in the dark for 30 minutes at room temperature. The reaction was stopped with 1 mL of HCl, 0.1 mol/L, and optical densities read in a ELISA Quantum II (Abbott) at 492.6 nm. The threshold optical density for a positive result was 0.065 for both serum and CSF.

RESULTS

Patients with ELISA positive in CSF had highest (optical density) values statistically different from both control groups ($p<0.001$). Only one

patient with ELISA negative and IH positive in CSF had (optical density) of 0.060 near cut-off point 0.065, this result could mean that this patient was in early state of CC when antibodies in CSF were not in high titer (Figure 1).

Two patients had negative results in CSF by both IH and ELISA. Patient 155 also was negative in serum by IH and ELISA, but the other was positive in serum by IH and negative by ELISA.

Figure 1 shows CSF ELISA results in patients with and without positive indirect hemagglutination results, and in control subjects. Figure 2 shows corresponding data for serum ELISA. Indirect hemagglutination in both serum and CSF had a 30% false negative rate (sensitivity 70%). Serum ELISA results had a similar false negative rate, but either CSF ELISA or IH was positive in all but 2 patients (sensitivity 90%). There were no false positive results in controls by either test.

DISCUSSION

Similar favorable results for ELISA were reported by Espinosa in Mexico (9) with 85% sensitivity for CSF and 73% for serum. Our micro ELISA needs small quantities of antigens, microliter quantities of sample, and small volumes of enzymatic reagents, rendering its cost less than previous ELISA methods (9). Capillary samples are sufficient for the serum test, an advantage for population screening. Its high sensitivity using CSF might reduce the need for expensive tests such as CT scan, or nuclear magnetic resonance (10), or invasive procedures such as angiography, pneumoencephalography or biopsy.

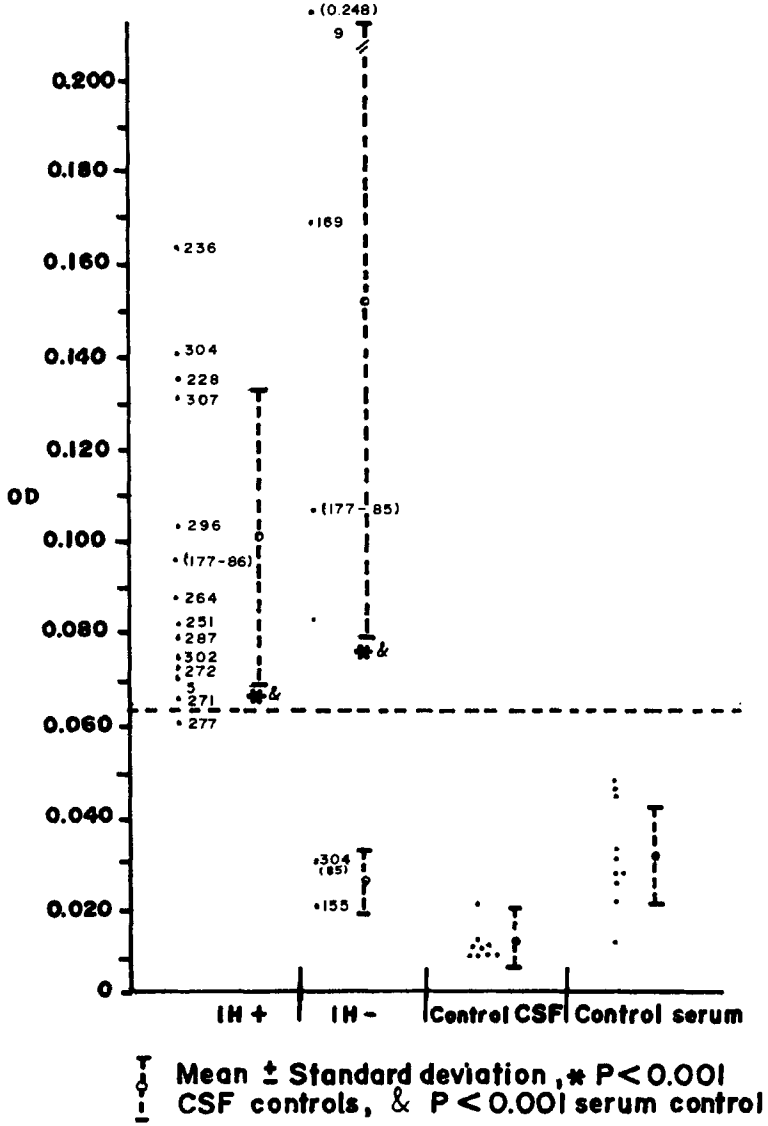

FIGURE 1: Results of ELISA (OD) in CSF of patients with CC compared to IH.

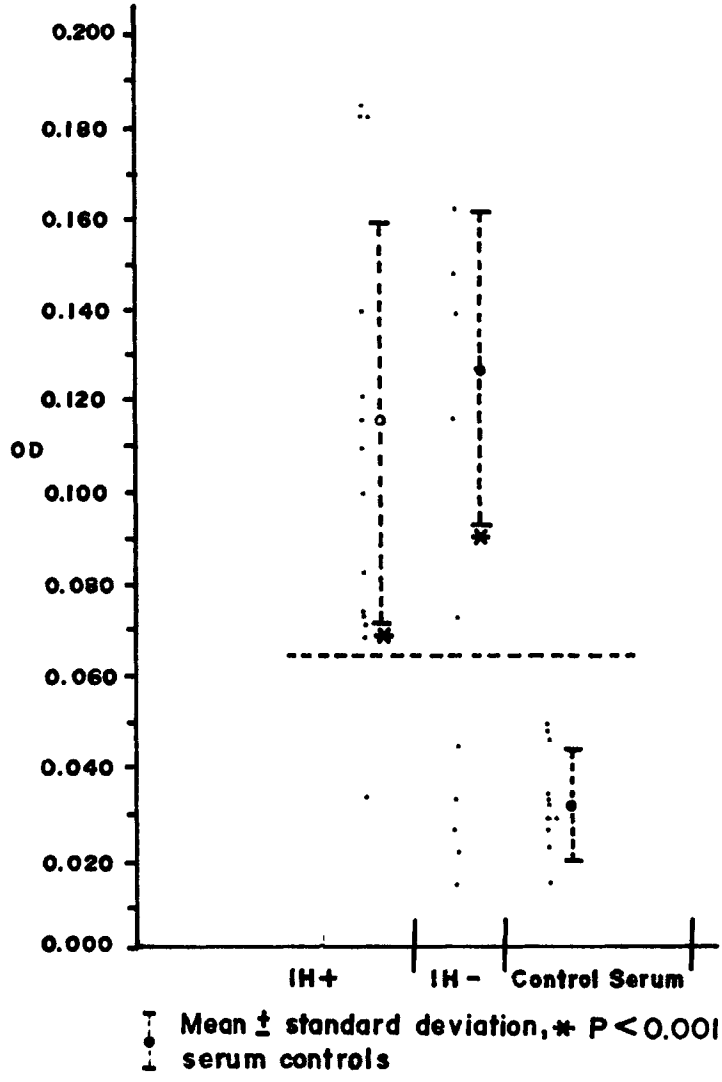

FIGURE 2: Results of ELISA (OD) in serum of patients with CC compared to IH.

The poorer diagnostic performance of indirect hemagglutination is not a result of a higher detection limit for antibody since there was no correlation between indirect hemagglutination titre and ELISA absorbance. The 2 tests must reflect the antigenic diversity of the infective organism. The cost of the ELISA test is less than 15 U.S. cents or 45 Colombian pesos which makes it acceptable for developing countries.

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