CIRCULATING PARASITE ANTIGEN IN PATIENTS WITH HYDROCEPHALUS SECONDARY TO NEUROCYSTICEROSIS


Abstract. End stages of neurocysticercosis include residual intraparenchymal brain calcifications and hydrocephalus. Although brain calcifications alone have a benign prognosis, hydrocephalus is frequently associated with chronic inflammation and intracranial hypertension, together with a protracted clinical evolution, and may lead to patient deaths. By using a monoclonal-based antigen detection enzyme-linked immunosorbent assay, we measured the levels of circulating parasite antigen in the sera of 56 patients with neurocysticercosis: 27 with calcifications only and 29 with hydrocephalus. The assay gave positive results in 14 of 29 patients with hydrocephalus but was consistently negative in patients with calcifications. Circulating parasite antigen in hydrocephalus secondary to neurocysticercosis indicates the presence of live parasites in these patients and thus a potential benefit from antiparasitic therapy.

INTRODUCTION

Infection of the human central nervous system (CNS) by the larvae of Taenia solium (neurocysticercosis, NCC) is endemic in most developing countries, where it is a major cause of seizures and other neurological morbidity. The disease is now also increasingly recognized in the United States and other industrialized countries largely as a result of immigration of tapeworm carriers from endemic areas.

The natural evolution of human neurocysticercosis is poorly understood. In more than half of cases, medical care is sought only after all viable parasites have died or cannot be detected; brain calcified scars in parenchymal neurocysticercosis and, less frequently, hydrocephalus in ventricular/subarachnoid cysticercosis. Both these conditions are considered “inactive” sequelae of the disease, although they have contrasting clinical evolution and prognosis. Thus, most patients with calcifications experience complete remission of symptoms or occasional seizures, whereas a torpid, progressive evolution or even death may occur in many hydrocephalus patients.

Disease progression in ventricular/subarachnoidal cysticercosis is associated with chronic inflammation around parasitic membranes or blockage of the circulation of cerebrospinal fluid (CSF) by a cyst.

Our group has applied in clinical settings a monoclonal antibody (HP10) that reacts with a repetitive carbohydrate epitope found in excretory/secretory and surface antigens of Taenia saginata. The test’s specificity, systematically evaluated in samples from with a wide range of helminthic and protozoa infections, has demonstrated remarkably low levels of cross reactions with other parasites as well as with normal human serum and CSF samples. An important characteristic of the HP10 epitope is its extreme resistance to degradation, whether it is caused by exposure to high temperature (3 days at 28°C, 1 hour at 57°C), repeated freeze-thaw cycles, or bacterial or yeast contamination. The assay was performed with slight modifications from the original report as follows: Microplate wells (Immulon 1, flat bottom, Dynatech Laboratory) were coated with 100 µL (10 µg/mL) of 50% saturated (NH₄)₂SO₄ precipitate of HP10 monoclonal antibody to T. saginata in carbonate buffer (pH 9.6) and left overnight at 4°C. Unbound antibody was removed by washing with normal saline 0.05% (w/v) Tween 20. Free binding sites were blocked with 1% (w/v) bovine serum albumin (BSA) diluted in phosphate-buffered saline (PBS) pH 7.3 with 0.05% (w/v) Tween 20 for 1 hour at room temperature. Undiluted serum samples (100 µL per well) were added and incubated for 30 min at 37°C. Then 100 µL/well of biotin-conjugated.
HP10 MAb diluted 1/2000 in PBS/BSA/Tween was added and incubated for 30 min at 37°C. Streptavidin-peroxidase conjugate (Pierce Ltd) was added, diluted 1/10,000 (1.0 mg/ml) in PBS/BSA/Tween, 100 μL/well, and the plates were incubated for 30 min at 37°C. At this point, 100 μL of TMB substrate (SIGMA Ltd) was added to each well, and plates were incubated for 15 min at room temperature. The reaction was stopped by adding 100 μL 0.2 M H2SO4 and read at 450 nm on a TitertekMultiskan. A sample was considered positive if the specific OD value was greater than the mean values of the negative control samples plus three standard deviations.

EITB tests were performed as originally described17 using 10 μL of serum. Positive identification was based on visualizing T. solium-specific antibodies reacting to any one of seven purified T. solium glycoprotein antigens (GP-Ags, diagnostic bands GP50, GP42–39, GP24, GP21, GP18, GP14, and GP13; the number indicates molecular weight in kilodaltons).

**Analysis.** Differences between the proportion of Ag-ELISA positive cases between groups were evaluated by chi-square test. Normality of optic density values was verified by one-sample Kolmogorov-Smirnov test, and Student’s t test was used to demonstrate differences in OD values if the underlying distribution was normal. Nonparametric Spearman’s correlation analysis was used to evaluate the association between number of reacting bands on EITB and after antigen levels on Ag-ELISA.

**RESULTS**

**Patients with calcifications alone.** Samples from 27 patients (10 males and 17 females; age range, 17–75 years; mean, 31.11 ± 9.70 SD) with calcified neurocysticercosis were included. All samples from this group tested negative in Ag-ELISA (mean OD, 0.023 ± 0.008; range, 0.009–0.045).

**Patients with hydrocephalus.** Samples from 29 patients (16 males and 13 females; age range, 23–77 years; mean age, 43.76 ± 14.64 SD) with hydrocephalus secondary to NCC were included. Almost half of them (14/29, 48.3%) tested positive on Ag-ELISA (P < 0.001 compared with the calcifications group, Fisher’s exact test). The mean OD for the group was 0.289 ± 0.326 (range, 0.014–0.924).

In the hydrocephalus group, 13 patients also had parenchymal calcifications. Eight of them were Ag-ELISA positive compared with only six of 16 patients with hydrocephalus but no parenchymal calcifications (P = 0.36, chi-square test). Patients with hydrocephalus and calcifications had higher values of Ag-ELISA compared with the remaining 16 patients (mean OD 0.429 ± 0.368 vs. 0.175 ± 0.242, P = 0.034 on Student’s t test) (Figure 1).

**Comparison of results from Ag-ELISA and EITB.** Only five samples (18%) from patients with calcifications showed antibodies to all the seven GP-antigen bands, whereas four (14%) reacted to a single band. There was no correlation between number of bands on EITB and OD levels in this group.

In patients with hydrocephalus, most cases (19/29, 65%) reacted to all the seven GP antigens on EITB. The median number of reactive bands on EITB was six; only one case (3%) reacted to a single band. There was a positive correlation between antigen levels and number of bands recognized on EITB (Spearman’s correlation coefficient 0.623, P < 0.01).

**DISCUSSION**

Neurocysticercosis is a frequent condition in most developing countries,19 causing much neurological morbidity and some mortality.1,3–7,10,11,15,20 The long-term prognosis of such patients is one of the less documented areas of clinical experience. The main sequelae of NCC are residual brain calcifications and hydrocephalus. Parenchymal calcifications are associated with seizures, which are easily controlled by antiepileptic drugs and so receive scarce attention as an “inactive” condition.5,7,8 Conversely, hydrocephalus is a known marker of poor prognosis.6 It may occur because of several different mechanisms that obstruct CSF pathways: intraventricular cysts, ependymitis, residual arachnoidial fibrosis, or compression by a neighboring intraparenchymal cyst.9,21 In some cases, hydrocephalus is well controlled with CSF shunting, whereas in others there are disease exacerbations, shunt blockage, and elevated mortality,4–11 even when cysts are not detectable on CT.22 We explored the presence of circulating parasite antigen in patients with “resolved” cysticercosis to assess the presence of live parasites in patients with hydrocephalus compared with patients with parenchymal calcifications only. The study demonstrated that a significant proportion (approximately half) of hydrocephalus patients present with high levels of circulating parasite antigen.

The Ag-ELISA uniquely detects living parasites because it is specific for a secreted antigen from viable metacestodes.12–14,19 Therefore, the most probable explanation for those patients with a positive assay is the presence of live parasites or parasitic membranes in the ventricular cavities or in the basal cisterns that were not detected by CT. Magnetic resonance imaging (MRI) will probably detect ventricular or basal parasites not seen on CT, but this is not always easily available23,24 and, even where available, is economically out of reach for most patients. Antigen-positive patients may be selected for this more expensive radiological procedure. Alternatively, hy-
drocephalus may coexist with viable cysticerci outside the nervous system in these patients. This seems unlikely because parasites located outside the CNS die years before those in the brain,25 probably because of accessibility to the immune response of the host. Hydrocephalus in Ag-ELISA-negative patients probably represents sequelae of the infection without surviving parasites.

All patients with parenchymal calcifications but not hydrocephalus were antigen negative. Whether it is to be expected that some antigens can be damaged by long-freeze storage of archive samples, previous studies with this assay have not shown any significant changes in test results. Inflammatory signs (edema and contrast enhancement) have been detected around already calcified cysticerci using MRI.26 This phenomenon is poorly detected by CT, and thus we can not exclude its presence in the patients in this study. If such inflammation is a response to antigenic stimulation, the most probable stimulus would be somatic parasite antigens released from the dead calcified cysts.

One of the major therapeutic interventions in NCC is the use of antiparasitic drugs, either praziquantel or albendazole.27–30 Evidence provided in recent years strongly supports that antiparasitic drugs are effective in killing subarachnoid and ventricular cysticerci.22,31–33 The antigen-positive hydrocephalus patients may potentially benefit from antiparasitic therapy, although a warning must be issued to carefully exclude the presence of cysts in the fourth ventricle or in other locations where the inflammatory reaction secondary to parasite death may cause life-threatening acute CSF blockage.34 Further monitoring of antigen levels may help to evaluate efficacy of antiparasitic therapy.14

Acknowledgments: Other members of the Cysticercosis Working Group in Peru include S. C. M. Martinez, S. Montano, J. M. Martinez, and H. Saavedra (Instituto de Ciencias Neurologicas, Lima, Peru), M. Verastegui and G. Herrera (Universidad Peruana Cayetano Heredia, Lima, Peru), C. Gavidia and N. Falcon (Universidad Nacional Mayor de San Marcos, Lima, Peru), and C.A.W. Evans (Cambridge University, England, UK). We are indebted to the personnel of the Laboratory of Cysticercosis of the ICN for help in sample management and data collection.

Financial support: This study was funded in part by the INCO-DC program of the European Union (grant CT95-0002), Food and Drug Administration (FD-R-001107), and National Institutes for Health (U19-A145431, TW00598, and RO3-A1-42037). The authors also wish to acknowledge the British Council for support in the form of a Higher Education Link Project between the University of Edinburgh and the Universidad Nacional Mayor de San Marcos (to A. E. Gonzalez and L. J. S. Harrison).

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