

CLINICAL IMMUNODIAGNOSIS OF NEUROCYSTICERCOSIS : THE SINGLE CYST CHALLENGE

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Abstract. Neurocysticercosis is predominantly a multilesional disease in most endemic countries but presents mainly as a solitary cysticercus granuloma (SCG) in India. Currently a diagnosis of neurocysticercosis is based on clinical and radiological criteria. Serological tests that confirm diagnosis measure the humoral immune response in patients and require introduction in India. Immunoassays detect multilesional neurocysticercosis at > 90% while SCG are detected in 40-60% of cases. This paper reviews our evaluation of several immunoassays in testing for SCG in Indian patients and attempts to improve sensitivity by antigen refinement.

INTRODUCTION

Neurocysticercosis (NCC), the infection of the central nervous system with the cysticercus of *T. solium*, is common in India and a major cause of neurological morbidity (Singh, 1997; Murthy and Yangala, 1998; Rajshekhar and Chandy, 2000). In endemic countries of Latin America, Africa, and most of Asia, the disease is mainly multi-lesional. In India, solitary cysticercus granulomas (SCG) contribute to almost 70% of the disease while multi-lesional NCC (MNCC) is seen in 30% of cases (Wadia *et al*, 1987; Rajshekhar and Chandy, 2000).

In most hospitals in India, NCC is diagnosed on clinical and radiological findings of the CT scan or MRI. It would be an advantage to include serological tests in the diagnostic decision of NCC to confirm diagnosis. Serological tests established for NCC detect MNCC at > 90% while the detection of solitary lesions of cysticercosis is reported to be < 50% (Chang *et al*, 1988; Ahuja *et al*, 1989; Wilson *et al*, 1991; Rajshekhar and Oommen, 1997). Clearly, the serodiagnosis of solitary lesions needs improvement. This paper discusses our experience with serological tests for SCG in patients who presented to the Neurology Services of the Christian Medical College Hospital with recent onset of seizures and radiological findings on a CT scan or MRI indicative of NCC.

MATERIALS AND METHODS

Patients

Patients whose blood was sent to the laboratory for routine cysticercosis serology were included in the analysis. Sera were stored at -20°C until assay. All patients diagnosed to have SCG (Rajshekhar and Chandy, 1997) or MNCC (Del Brutto *et al*, 2001) had a contrast enhanced CT or gadolinium enhanced MRI of the brain.

ELISA for detection of circulating cyst antigen (Ag-ELISA)

Ag-ELISA for the detection of circulating serum *T. solium* metacestode antigen using monoclonal antibodies to excretory / secretory products of *T. saginata* metacestodes was carried out as established by Brandt *et al* (1992) and modified by Dorny *et al* (2000). The cut-off was determined from the mean absorbance of 8 negative control sera +3 standard deviations. An ELISA ratio was then calculated by dividing the absorbance of the sample by the calculated cut-off value. Samples with ELISA ratios ≥ 1 were considered positive for cyst antigen. Seventy sera from SCG patients, 30 sera from MNCC patients, and 100 non-cysticercosis sera were estimated for circulating cyst antigens.

Immunoassays using isoelectric focussed (IEF) *T. solium* metacestode proteins

IEF purified *T. solium* cyst (from Ecuador and from Vellore) antigens were prepared as given by Ito *et al* (1998). ELISA for detection of cysticercal antibodies was carried out with these antigens at 1 µg/ml and sera diluted 100 fold (Ito *et al*, 1998). A sample was considered positive in the ELISA > mean absorbance +1 standard deviation of 26 negative controls. Forty-

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one sera of solitary cysticercus granuloma patients, 22 sera of multiple neurocysticercosis and 26 non-cysticercosis sera were analyzed by ELISA using these proteins as antigens. The isoelectric focussed proteins (1 mg/ml) were used in immunoblots for cysticercal antibody detection as given by Ito *et al* (1998). A serum sample was considered positive for cysticercosis if one or more bands < 50 kDa were detected on the blot. Forty-one sera of solitary cysticercus granulomas, 24 sera of multiple neurocysticercosis and 26 negative controls were analyzed on immunoblots using these proteins.

EITB

T. solium cysts from infected pigs in Vellore were extracted with urea and extracts purified for lentil lectin specific glycoproteins according to the method of Tsang *et al* (1989). Using these proteins as antigens, sera were analyzed for cysticercosis by the EITB (Tsang *et al*, 1989). Forty-five sera from patients with SCG, 12 sera from patients with MNCC, and 13 negative controls were assayed by the EITB.

Immunoassays using non-solubilized lentil lectin specific *T. solium* metacystode glycoproteins

Lentil lectin specific glycoproteins extracted from *T. solium* metacystodes without detergent as given by Prabhakaran *et al* (2004) were used in a cysticercal antibody ELISA at 1 µg/ml with sera diluted at 1:100. A sample was considered positive for cysticercal antibodies with an absorbance above the mean absorbance of 100 non-cysticercosis sera +2 standard deviations. These proteins were used in immunoblots on 4-20% SDS-PAGE (250 ng/mm gel) with sera diluted 100 fold as reported earlier (Prabhakaran *et al*, 2004). Blots were considered positive for cysticercosis on appearance of 1 or more bands of <50 kDa. One hundred and seven sera from patients with SCG, 43 from patients with MNCC and 100 non-cysticercosis sera were analyzed by these tests. SDS-PAGE (4-20% gels) was carried out by the method of Laemmli (1970) and gels stained for protein with silver by the method of Merrill *et al* (1983).

RESULTS

Ag-ELISA

Sera of 7 out of 70 SCG patients, 15 of 30 MNCC patients, and 5 of 100 non-cysticercosis patients were positive for circulating cyst antigens.

Immunoassays with IEF cyst proteins

Seventeen of 41 sera from SCG patients and 20 of 22 sera from MNCC patients were positive for

cysticercal antibodies on the ELISA using IEF antigens. Non-specific cross-reactions with these antigens were seen in 4 of 26 non-cysticercosis sera. On immunoblots using IEF antigens, 7 of 41 sera from SCG patients and 15 of 24 sera from MNCC patients were positive for cysticercosis. Non-specific, cross, reactions were noted in 2 of 26 sera from non-cysticercosis patients.

EITB

Sera of 27 out of 45 SCG patients, 11 of 12 MNCC patients, and 1 of 13 non-cysticercosis patients were positive by the EITB for neurocysticercosis.

Immunoassays using non-solubilized lentil lectin specific *T. solium* metacystode glycoproteins

Eighty-six of 107 sera from SCG patients, 41 of 43 from MNCC patients, and 6 of 100 from non-cysticercosis patients were positive for cysticercal antibodies on the ELISA using *T. solium* proteins extracted from whole cysts without detergent. Using these proteins in immunoblots, 67 of 107 sera from SCG patients, 37 of 43 from MNCC patients, and 3 of 100 from non-cysticercosis patients were positive for cysticercosis.

The diagnostic parameters of the different assays for SCG detection in the patient population tested are given in Table 1.

DISCUSSION

A major requirement of serological testing for NCC in India is to detect SCG, the predominant form of the disease in the country. Several studies have reported a poor sensitivity in the serodiagnosis of solitary NCC lesions and suggest that the antigenic challenge from a solitary cyst is possibly too low to be detected. Our studies partially support this statement because circulating cyst antigens were seen in only 10% of patients with SCG but in 50% of patients with MNCC. Our experience with serological tests for SCG using differently processed *T. solium* metacystode antigenic proteins is that on immunoblots solitary lesions are detected in 60% of patients while detection with the more sensitive immunoassay of ELISA is 80% : this was with *T. solium* glycoproteins extracted from metacystodes without the use of chaotropes/detergents. An 80% sensitivity for a diagnostic test for SCG is inadequate and efforts to improve the detection rate of SCG continue. The 94-97% specificities of these immunoassays for NCC also need to be evaluated as the cross-reactions arise mainly from patients with brain neoplastic lesions. Lentil lectin specific

Table 1
Serodiagnosis of solitary cysticercus granulomas in Indian patients.

Antigen	Method	Sensitivity (%)			Specificity (%)
		SCG	MNCC	AII NCC	
Lentil lectin glycoproteins (<i>T. solium</i> - Vellore)	EITB	60.0	91.7	66.7	92.3
IEF glycoproteins (<i>T. solium</i> - Ecuador)	Immunoblot	17.1	62.5	33.8	92.3
	ELISA	41.5	90.9	58.7	84.6
Lentil lectin glycoproteins (<i>T. solium</i> - Vellore)	Immunoblot	62.6	86.0	69.3	97.0
	ELISA	80.3	95.3	84.6	94.0

glycoproteins from metacestodes obtained from Peru were of similar diagnostic performance for SCG in the EITB as those from Vellore. IEF cyst proteins obtained from cysts from Vellore were also similar in test characteristics to those from Ecuador.

A comparison of the SDS-PAGE protein profiles of the different antigens in this study showed proteins of > 100 kDa interfered in the tests and that the fewer the proteins in this range the better the immunodiagnostic efficacy, especially the specificity, of the antigen. For Indian patients it was also noted that the concentration of cyst glycoproteins of 10-18 kDa in the antigen fraction contributed to the sensitivity and specificity of detection: high concentrations of these proteins increased the non-specific noise of the test.

From these studies we would suggest that although the antigenic challenge in patients with SCG is low, (with circulating cyst antigens detected in only 10% of these patients), their immune response is not too low to be detected, with a humoral response measurable in 80% of them.

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