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BRIEF COMMUNICATION

DETECTION OF *Cysticercus* ANTIGENS AND ANTIBODIES IN CEREBROSPINAL FLUID OF PATIENTS WITH CHRONIC MENINGITIS

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SUMMARY

Chronic meningitism is a less frequent manifestation of neurocysticercosis caused by *Taenia solium* cysticerci. In the present study we used Co-agglutination (Co-A), a simple and rapid slide agglutination test to detect specific *Cysticercus* antigen in the 67 cerebrospinal fluid (CSF) samples from patients with chronic meningitis of unknown etiology. The results were compared with that of ELISA for detection of antibodies. Among these samples four (5.97%) were positive for *Cysticercus* antigen by Co-A test and six (8.95%) were positive for antibodies by ELISA. Two samples were positive by both Co-A and ELISA, two were positive only by Co-A and four were positive only by ELISA. In the present study, although *Cysticercus* antigen and antibodies were present in CSF samples from eight (11.94%) patients, we cannot affirm that all the cases of chronic meningitis are due to cysticercosis, but for any case of chronic meningitis of unknown origin, it would be useful to consider the possibility of cysticercal meningitis.

KEYWORDS: Chronic meningitis; Neurocysticercosis; Cerebrospinal fluid; *Cysticercus* antigen; Anti-*Cysticercus* antibodies; Co-A test.

Neurocysticercosis (NCC) is one of the most common parasitic diseases of the central nervous system prevalent worldwide. The condition is caused by *Cysticercus cellulosae,* the metacestode larva of *Taenia solium*. In most of the developing countries, NCC comprises 10% of acute neurologic cases[7]. Epilepsy is the usual presentation of the condition. Other known presentations include raised intracranial pressure, meningoencephalitis, focal neurological deficit and psychiatric symptoms. Chronic meningitis is the chronic inflammation of the meninges where characteristic neurologic syndromes exist for > 4 weeks and is associated with a persistent inflammatory response in the cerebrospinal fluid. The entity of chronic meningitis as part of the spectrum of NCC although observed to be rare in comparison to other symptoms, nevertheless have been documented by several authors[8,14,15,24,25]. Headache, vertigo, vomiting, papilloedema, an altered level of consciousness, and gait disturbances may be present in patients with meningitis caused by *T. solium* cysticerci[21]. Thus diagnosis of NCC at the clinic is difficult because of variable clinical signs and symptoms and imaging findings are usually inconclusive or of poor specificity[5]. An EITB assay using lectin purified glycoprotein antigens has been reported to be 100% specific and 98% sensitive for diagnosis of NCC[26]. But the preparation of the antigen for the EITB assay is costly as well as difficult to perform. Moreover, these diagnostic kits need to be imported from the overseas. Hence there is necessity to develop reliable and economical in-house serological test for diagnosis of cysticercosis.

In the present study we used co-agglutination (Co-A), a simple and rapid slide agglutination test to detect *Cysticercus* antigen and ELISA to detect specific anti-C*ysticercus* antibodies in the cerebrospinal fluid, to establish a diagnosis of chronic meningitis of unknown etiology.

CSF samples collected from 67 patients with chronic meningitis were processed at the Department of Microbiology, JIPMER Hospital, Pondicherry. CSF samples from 12 patients with meningitis caused by *Hemophilus influenzae* (two), *Staphylococcus aureus* (two), *Streptococcus* sp. (two), *Enterococcus faecalis* (one), *Acinetobacter boumani* (two), *Pseudomonas* sp. (two) and *Cryptococcus neoformans* (one) were used as negative controls. The CSF samples were stored at -20 ^{0}C before testing.

Hyperimmune serum against *C. cellulosae* whole cyst antigen (total soluble protein antigen) was raised in white New Zealand rabbit in divided doses with Freund's complete adjuvant in the primary dose and with incomplete adjuvant in the booster doses. The IgG fraction from the immune serum was purified by precipitation with cold

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saturated ammonium sulphate by the method of GOTTSTEIN[9] as cited by DEVI & PARIJA[6].

ELISA and Co-agglutination (Co-A) were performed to detect specific *Cysticercus* antibodies and antigens, respectively, in the CSF samples. The standard procedure as described by CROWTHER[4] was followed with some modifications. Briefly, polystyrene microtiter plates (NUNC) were coated with the *C. cellulosae* whole cyst antigen (total soluble protein antigen) at the concentration 1 µg/100 µL/well. The non-specific binding sites were blocked by BSA (2%) in PBS 7.2. Optimum dilution of sample and standard CSF (CSF of confirmed positive patients) were prepared in sample dilution buffer (PBS 7.2 containing 0.05% Tween-20) at the dilution 1:50 and 100 µL of diluted CSF was added in duplicate. Goat anti-human-IgG-HRP conjugated secondary antibody (DAKO) was incubated at the dilution of 1:1000. The plate development was done with freshly prepared substrate solution (6 mg OPD in 10 mL of PBS pH 7.2 and 10 µL of 30% H_2O_2). The reaction was stopped by adding 50 µL of 2N H_2SO_4 in each well. Then the absorbance was read at 492 nm in an ELISA reader (TEKAN). CSF dilution and the antigen concentration that gave OD_{492} value of more than the *cut-off* (mean titre of negative CSF samples + 2 SD) were considered as positive.

The co-agglutination (Co-A) test for antigen detection was performed as per the procedure described by PARIJA & REDDY[18]. Twenty four hours grown cells of *Staphylococcus aureus* (Cowan I strain) bearing protein A (SAPA) cells were harvested into PBS pH 7.2 and centrifuged at 3000 X g for 10 minutes, followed by washing three times with PBS pH 7.2 containing 0.05% sodium azide (NaN_3). The pellet was fixed in 10 volume of 1.5% formaldehyde in PBS 7.2 at room temperature (RT) for 90 minutes, followed by washing three times. Then the cells were resuspended to 10 volume of buffer containing 0.05% NaN_3 and heated for five minutes at 80 ^{0}C followed by washing three times. Finally the cells were resuspended in PBS 7.2 containing 0.05% NaN_3. 0.1 mL of purified hyperimmune serum anti-*C. cellulosae* was added to 1 mL of 10% suspension of SAPA cells, mixed well and left at RT for about 30 minutes followed by washing two times. The cells were then resuspended to a concentration of 2% cells in PBS 7.2 containing 0.1% NaN_3 and these sensitized cells were stored at 4 ^{0}C till use. In parallel formalin fixed SAPA cells were incubated with normal saline instead of hyperimmune serum to get unsensitized SAPA cells as control cells.

The test was performed on the glass slides, with two aliquots of 25 µL of CSF sample. An equal volume (25 µL) of sensitized SAPA cell suspension was added to one aliquot and the same volume (25 µL) of a 2% suspension of unsensitized SAPA cells was added to the other. The slide was then rotated manually for two min and then observed. In a positive Co-A test, the addition of sensitized SAPA cells to the test CSF resulted in large visible clumps of cells within two min; in a negative reaction, no visible clumping was observed. Agglutination with the sensitized cells and not with the unsensitized cells was considered to be a positive result.

In the positive control test, 10 µL of stock *C. cellulosae* total soluble protein antigen (protein content 0.7 g/dL) was added to 90 µL of PBS pH 7.2 (1:10) and then diluted two fold up to 1:1280 with PBS pH 7.2. and taken as positive control. The visible clumping with sensitized cells and no agglutination with unsensitized cells showed valid test. The highest dilution of the antigen showing visible agglutination with sensitized cells and no agglutination with unsensitized cells was considered as the detection limit of the test. In the negative control test, instead of antigen, saline was taken for negative control test and there was no agglutination with either sensitized or unsensitized cells.

A total of eight CSF samples were positive for either *Cysticercus* antigen or antibodies by Co-A and ELISA, respectively. The results of 67 CSF samples tested by ELISA and Co-A are presented in the Table 1. The antigen detection limit of the Co-A test using the particular batch of reagent preparation was found to be up to 0.27 µg.

Table 1
Comparison of Co-A and ELISA results obtained with CSF samples collected from 67 patients with chronic meningitis of unknown origin

ELISA result	Co-A result		Total (%)
	No. (%) positive	No. (%) negative	
Positive	2 (2.98)	4 (5.97)	6 (8.95)
Negative	2 (2.98)	59 (88.05)	61 (91.04)
Total	4 (5.97)	63 (94.02)	67 (100)

The specific clinical diagnosis of chronic meningitis caused by cysticercosis is difficult. It is observed that cysticercal meningitis often mimics other forms of chronic meningitis[15,25] where serial CSF examination shows consistently raised protein, decreased glucose level and pleocytosis. In addition to signs of meningeal irritation, increased intracranial pressure due to inflammation, edema, or an obstructing cyst may be present[23].

Many reports of cysticercal aetiology of chronic meningitis have been documented from various parts of the World. In a recent study, meningitis was found to be a presenting symptom in 4.1% of NCC patients examined in Dehra Dun, India[27]. In a 12-year period data analysis of subacute to chronic meningitis cases from South India, anti-mycobacterial antibodies and anti-*Cysticercus* antibodies were found in 35.4% and 5.47% of the cases of NCC respectively[2]. NCC presenting as chronic meningitis is also being reported from Italy[12], Brazil[1], Portugal[16], Korea[3,28], and other endemic parts of the World[10,12,20,21].

Our present study showed that of 67 patients with chronic meningitis of unknown origin 5.97% were positive by Co-A, whereas, 8.95% were positive by ELISA. Among Co-A negative CSF samples, four were positive by ELISA. The failure of detecting antigen in these CSF samples could be due to the absence of antigen in the CSF, to antigen concentration that were below detection limit of the assay or to degradation of antigen during storage of CSF at -20 ^{0}C.

Serology of NCC may be resulting with apparently false positive tests for several reasons: some healthy subjects residing in endemic areas may have serum antibodies induced by previous infections that

did not progress to the establishment of cysticerci, or because they bear cysticerci that are localized in clinically inconspicuous anatomic sites[22]. Also a major cause of truly false positive serology in methods using whole or partially purified antigen preparations is the extensive sharing of antigenic epitopes of many cestodes and helminths[13]. But the extent of cross-reacting antibody in CSF is supposed to be less common than that in serum[17,19].

In the present study, we cannot affirm that all the cases of chronic meningitis are due to cysticercosis, but for any case of chronic meningitis of unknown origin, it would be useful to consider the possibility of cysticercal meningitis. This is important particularly in the area where both tuberculosis and NCC are endemic and make the clinical diagnosis of chronic meningitis most confusing. KATTI & ACHAR[11] have demonstrated both anti-mycobacterial and anti-*Cysticercus* antibodies in 17.14% of CSF samples from patients with chronic meningitis. Moreover, cysticercosis may be the underlying etiology associated with chronic meningitis cases especially when other infectious etiology is unknown.

RESUMO

Detecção de antígenos e anticorpos de *Cysticercus* em fluido cerebrospinal de pacientes com meningite crônica

Meningite crônica é manifestação pouco freqüente de neurocisticercose causada por cisticerco de *Taenia solium.* No presente estudo utilizamos co-aglutinação (Co-A) um teste simples e rápido de aglutinação para detectar antígeno específico de *Cysticercus* nas 67 amostras de fluido cerebrospinal (CSF) de pacientes com meningite crônica de etiologia desconhecida. Os resultados foram comparados com os de ELISA para detecção de anticorpos. Dentre estas amostras quatro (5,97%) foram positivas para antígenos de *Cysticercus* pelo teste Co-A e seis (8,95%) foram positivas para anticorpos por ELISA. Duas amostras foram positivas por ambos Co-A e ELISA, duas foram positivas somente por Co-A e quatro foram positivas somente por ELISA. No presente estudo embora antígenos e anticorpos de *Cysticercus* estivessem presentes nas amostras de CSF de oito pacientes (11,94%), não podemos afirmar que todos os casos de meningite crônica sejam devidos à cisticercose, mas para qualquer caso de meningite crônica de origem desconhecida seria útil considerar a possibilidade de meningite por cisticerco.

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