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Case Report

CYST FLUID ANTIGEN BASED ENZYME LINKED IMMUNOELECTRO TRANSFER BLOT (EITB) FOR DIAGNOSIS OF A CASE OF NEUROCYSTICERCOSIS

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ABSTRACT

Enzyme linked immunoelectro transfer blot (EITB) is considered as an important serological test for diagnosis of neurocysticercosis (NCC); though the test needs further validation in taeniasis endemic area.

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INTRODUCTION

Neurocysticercosis (NCC), the most common parasitic infection of the central nervous system (CNS) is an important neurological disorder throughout the world. NCC caused by the larval stage of *Taenia solium* is the single most common cause of seizure/epilepsy in the developing countries(1). Human is the only definitive host of *T. solium* harboring the adult tapeworm in the intestine (taeniasis), while both human and pig can act as intermediate host and harbor the larvae in different internal organs (cysticercosis) including brain (neurocysticercosis).

Diagnosis of NCC is impaired by the polymorphic and nonspecific symptoms, and the presence of clinical and radiological findings that are similar to other diseases of the CNS (2, 3). Hence, the International Working Group on NCC recommended clinical, neuroimaging, immunological and epidemiological criteria for definitive and probable diagnosis of NCC. Neuroimaging is considered to be the most specific tool available for diagnosis of NCC(1). MRI may occasionally fail to detect calcified cyst, and CT may not always be specific for viable/degenerative cysts. Moreover, these techniques are expensive and not readily available in developing countries, especially in rural endemic areas.

Detection of anti-cysticercal antibodies in serum or cerebrospinal fluid (CSF) by enzyme linked immunosorbent assay (ELISA) or immunoblot is an important diagnostic tool for NCC. Detection of specific antibodies circulating in the sera or CSF by ELISA or immunoblot is helpful in confirming or excluding the diagnosis (4). Any band of ≤ 50 kDa recognised are considered to be positive for cysticercosis (5). The difference in recognition pattern of EITB bands has been reported in literature from different parts of the world. In a study from latin America, cyst antigens of >24 kDa were most commonly detected in NCC patient (6), while in Indian patients antigens ≤ 18 kDa were most commonly recognized (7). *Sato et al. 2006* reported that the glycoproteins (GPs) isolated from different genotypes of *T. solium* (i.e. African/American and Asian), showed different band patterns in EITB when probed with polyclonal or monoclonal antibodies raised in rabbit (8). Moreover, patients with taeniasis are also reported to be seropositive on EITB (9). Studies evaluating EITB as a diagnostic tool for NCC in endemic communities with antigens prepared from cysts of the same endemic area are lacking. Besides, preparation of antigens for EITB as originally described is complicated, expensive and not readily available in developing countries (5). A cyst fluid antigen based EITB was done in the index patient to look for the presence of specific antibody against *T. solium*.

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Case Report

After only one episode of seizure, a 24 year old man was started with phenytoin 3 years back. He had no recurrence of seizure neither had undergone any investigation after that. He was a pork consumer who later converted himself to a vegetarian. After around 3-4 months he developed depressive symptoms for which he sought treatment from a psychiatrist and recovered. He was referred to neurology OPD for opinion regarding seizure. His CSF and serum specimen was collected and subjected to a cyst fluid antigen based enzyme linked immunoelectro transfer blotting (EITB), the details of which is presented here.

Enzyme linked electro-immune transfer blot (EITB)

The fluid aspirated from muscle cysts dissected from infected pig was used as source of antigens for EITB. The fluid was sonicated at 20 kHz for 4 minutes of 1 minute pulse each followed by centrifugation at 200,000 x g for 2 hrs. Supernatant was separated and protease inhibitor cocktail (Sigma-Aldrich, MO, USA) was added and stored at -80° C till further use.

On a SDS-PAGE (15%) mini gel, 40 microgram of prepared antigen was loaded on vertical electrophoresis cell. The separated proteins were blotted on nitrocellulose membrane (Sigma- Aldrich, MO, USA) at 20V for 6 hrs. The membrane was cut into strips after blocking with 5% skimmed milk. One strip each incubated with the patient's serum (1:100 dilutions) and his CSF (Undiluted) at 4°C overnight. Following washing with phosphate buffered saline, the strips were incubated with HRP labeled anti-human goat IgG (1:1000 dilutions) (Sigma-Aldrich, MO, USA) for 3 hrs with constant shaking at room temperature. Diaminobenzidine (DAB, Bangalore Genei, India) was used for the development of the blots and analyzed. Three different bands were identified, which were similar both in serum and CSF (Fig. 1).



Fig. 1 EITB Strip 1-Serum Strip 2- CSF

DISCUSSION

Initially when EITB was introduced by CDC, Atlanta, it was considered to be a test with high sensitivity and specificity (5). But subsequent studies showed low sensitivity of the test especially in patients with single cyst infection (10). In Ecuador, the overall sensitivity was found to be 53.6% (11). It has been reported that patients with taeniasis are also seropositive on EITB thus questioning its diagnostic value for NCC in highly taeniasis endemic population (9). Cyst fluid antigen based EITB is considered to be specific and less cross reactive with other parasitic and nonparasitic diseases on immunoblot (12).

Ito *et al.* (1998) had reported that antigenic fractions from cyst fluid contained at least three major bands ranging from 10 to 26 kDa that were the most specific for differential diagnosis of NCC (12). However, the EITB based on semi-purified antigens are prone to more background noise and cross reactivity.

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