UTILITY OF CYSTICERCUS FASCIOLARIS ANTIGEN IN DOT ELISA FOR THE DIAGNOSIS OF NEUROCYSTICERCOSIS

NITIN SHUKLA, NUZHAT HUSAIN, GIRDHAR G AGARWAL, MAZHAR HUSAIN

ABSTRACT

BACKGROUND: Clinical diagnosis of neurocysticercosis (NC) is established by CT scan and MRI. However, absolute diagnosis is not possible in a fair number of cases, and serological assays are used as adjunct. Besides, CT scan and MR imaging are resource-intensive tests and not practical for screening in endemic areas. AIM: To provide a low-cost, efficient, and reproducible assay for the detection of antibodies against cysticerci. Hence we have attempted to standardize and evaluate the diagnostic utility of the cysticercus fasciolaris antigen in a Dot ELISA assay for diagnosis of NC. SETTING AND DESIGN: Tertiary hospital–based, case-control series. MATERIALS AND METHODS: Confirmed cases of NC diagnosed by presence of ring lesions in CT scan or MR imaging were taken as positive controls (n = 50). Negative controls (n = 50) included subjects with normal CT scan studies (n = 30) and diseased controls with ring lesions in CT scan confirmed to be neurotuberculosis (n = 20). Dot ELISA was standardized and validated with commercially available ELISA (UBI, USA) using sera from the study groups. STATISTICAL ANALYSIS: Chi-square test was used to compare the immunodiagnostic performance of the two tests. P value less than .05 (P <0.05) was considered significant. RESULTS: The Dot ELISA had a sensitivity of 88% and specificity of 74% with a positive predictive value of 77.19% and negative predictive value of 81.06%. Likelihood ratios for a positive and a negative test were 3.4 and 0.2. The sensitivity and specificity of commercial ELISA were 92% and 84% respectively. Difference between the performances of the two tests was not significant statistically. CONCLUSIONS: Dot ELISA has sensitivity and specificity comparable to ELISA for the diagnosis of NC. The test is simpler, not requiring expertise and instrumentation. Further validation of the test as a screening tool is required.

Key words: Cysticercosis, Dot enzyme-linked immunosorbent assay (ELISA), ELISA

INTRODUCTION

Neurocysticercosis, an infestation caused by lodging of larval stage of *Taenia solium* in the brain, is the most common parasitic disease of the central nervous system. It is reported...
to cause 50,000 deaths per year throughout the world.[1] The disease is endemic in Andean area of South America, Brazil, China, Indian subcontinent, Indonesia, Mexico and Central America, Southeast Asia, and Sub-Saharan Africa. The diagnosis of neurocysticercosis is based on CT scan and MR imaging where the lesions are visualized as single or multiple ring-enhancing lesions. A set of diagnostic criteria have been listed with certain degrees of certainty by Del Brutto.[2] Visualization of scolex of the parasite in cystic lesion is an absolute diagnostic criteria in CT scans or MR imaging. The scolex may not, however, be visualized in many cases due to varying levels of imaging. The differential diagnosis of cystic ring lesions in CT varies from inflammatory conditions like tuberculosis, toxoplasmosis, and brain abscess to small gliomas and arteriovenous malformations. Serological tests like ELISA and EITB are used as an adjunct to confirm the diagnosis.

Most immunodiagnostic tests utilize membrane or scolex antigens or their purified derivatives from cestodes of Taenia species. We have used membrane antigen of Taenia taeniaeformis (larval stage of Taenia taeniaeformis pathogenic in rat) in anti-IgG and anti-IgM ELISA for the immunodiagnosis of NC.[3] In the current study, we have designed and evaluated a Dot ELISA using membrane extract of cestodes of Taenia taeniaeformis antigen for immunodiagnosis of neurocysticercosis.

**MATERIALS AND METHODS**

**Study setting**
Tertiary hospital–based study.

**Study design**
Case-control study for diagnostic test evaluation.

**Study sample**
Fifty confirmed cases of NC (n = 50) with ring-enhancing lesions in which scolex was visualized in CT scan or MR imaging were used as positive controls. Of these, 36 cases had a single cyst and 14 had multiple cysts in the parenchyma. Age- and sex-matched 50 negative controls included 30 cases with normal CT scan (undergoing the test for any other indication) and 20 diseased controls with CT ring lesions confirmed to be neurotuberculosis by MRI studies and MR spectroscopy.[4] Cases with normal CT scan did not have any clinical evidence of cysticercosis in the form of epilepsy or subcutaneous nodules. Mean age of cases was 22.86 ± 12.68 years; and controls, 22.83 ± 16.02 years. Four milliliters of blood was taken from the subjects of both the groups under aseptic precautions and allowed to clot. The test sera thus collected was aliquot and kept at 70°C for future use. Both groups were screened for parasites in the stool examination. Institutional ethical clearance was obtained for the study setting.

**Experimental techniques**

**Preparation of antigen:** The eggs from gravid segments of Taenia taeniaeformis which were obtained from intestines of cats were gently separated and suspended in normal saline at a concentration of 100 eggs/mL. Rattus rattus were orally infected with 0.2 mL of the egg suspension. After 60 days the rats were sacrificed, cysticercus fasciolaris cysts were dissected from the liver on ice, membranes were separated from the scolex and washed with cold phosphate buffered saline (PBS) at pH 7.4. The membranes thus separated were homogenized in five volumes of PBS, sonicated three times at 30S, and cold-centrifuged for 30 minutes at 20,000 g. The concentration of protein was measured by Lowry’s method. The supernatant at a protein concentration of 150 mg/dL was used as the concentrated antigen.

**Dot ELISA:** One microliter of antigen (15 µg/mL) was dotted on to a square strip of nitrocellulose membrane 2×2 cm in size. The strips were air-dried for 10 minutes. The strips were then blocked with 2% bovine serum albumin in PBS for 2 hours over a shaker, followed by three washings with 0.2% Tween 20 in PBS (pH 7.4) over a period of 15 minutes. All incubations were done in plastic ice trays. The strips were incubated at room temperature with test serum diluted (1:2000) in 0.2% Tween 20 in PBS (PBST), for 60 minutes on a shaker. Three washings were repeated. Rabbit antihuman IgG-peroxidase conjugate (Dakopatts, Denmark) was added at a dilution of 1:2000 in PBST and incubated for 30 minutes over the shaker and then washed thrice at the end. At the end, diaminobenzidine (6 mg in 5 mL of PBST with 30 µL of hydrogen peroxide) was added. The strips were then incubated for 10 minutes in the dark. Reaction was stopped with distilled water. A deep brown–colored dot was considered as reactive, whereas no dot was considered as nonreactive. The serum samples were tested in a single-blind manner. The test was repeated on two different occasions to check for reproducibility, which was 100%. Blocked strips with antigen and other reagents were stored at 4°C.

**ELISA:** Commercial ELISA (UBI, USA) was used according to the manufacturer’s instructions. The test detected presence of IgG antibodies to T. solium cysticerci.

**Statistical analysis**
Validation and consistency checks were carried out. Sensitivity, specificity, and predictive values and likelihood ratios for Dot ELISA and commercial ELISA in the diagnosis of neurocysticercosis were calculated. Chi-square test was used to compare the immunodiagnostic performance of the two tests. P value less than 0.05 was considered significant.

**RESULTS**

**Dot ELISA**
Dot ELISA was positive in 44/50 (88%) radiologically proven NC cases and 13/50 (26%) negative controls (8/30 with normal CT scan and 5/20 with neurotuberculosis). Six radiologically proven cases that tested negative had single cysts. Overall the Dot ELISA was found to be 88% sensitive and 74% specific with a positive predictive value of 77.19% and negative predictive value of 81.06%. Likelihood ratios were 3.4 for a positive and 0.2 for a negative test [Table 1].

**Commercial ELISA**
ELISA detected 46 of 50 positive controls, while 4/50 could not be detected and were negative. These four cases were also negative in the Dot ELISA. Forty-two negative controls were test negative, while 8/50 were test positive.
Table 1: Performance characteristics of Dot ELISA for IgG and commercial enzyme-linked immunosorbent assay (ELISA) compared with CT scan (gold standard)

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity (95% C.I.)</th>
<th>Specificity (90% C.I.)</th>
<th>Positive predictive value (95% C.I.)</th>
<th>Negative predictive value (95% C.I.)</th>
<th>Positive Test</th>
<th>LR for Negative Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dot ELISA</td>
<td>88.0 (70.9, 97.0)</td>
<td>74.0 (62.0, 86.0)</td>
<td>77.2 (66.8, 88.0)</td>
<td>81.6 (76.0, 96.0)</td>
<td>3.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Commercial ELISA</td>
<td>92.0 (84.0, 99.5)</td>
<td>84.0 (73.8, 94.2)</td>
<td>85.2 (75.7, 94.7)</td>
<td>91.3 (83.2, 99.4)</td>
<td>5.8</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Table 1: Performance characteristics of Dot ELISA for IgG and commercial enzyme-linked immunosorbent assay (ELISA) compared with CT scan (gold standard)

The differences between the values of sensitivity and specificity of the two tests were not found to be significant (P value for sensitivity = 0.5; P value for specificity = 0.2) [Table 1].

**The Discussion**

ELISA and EITB are the most commonly used serological tests for cysticercosis but require equipment and trained personnel and are not practical for use in screening in peripheral laboratories. Owing to the limitations of ELISA and EITB and problems faced in raising antigen in bigger mammals like pigs, there is a need for an easier, cheaper test format using alternate source of antigen. Hence the current study was undertaken to design and evaluate the efficacy of Dot ELISA using cysticercus fasciolaris antigen in immunodiagnosis of cysticercosis.

The cysticercus fasciolaris antigen, used in the study, can be produced in Rattus rattus under standardized conditions within a short duration of 60 days and can be a practical alternative to the commonly used cellulose antigen requiring longer development time and maintenance of a bigger animal (pig). We have evaluated diagnostic efficiency of experimental ELISA using cysticercus fasciolaris antigen and IgG and IgM antibodies and observed an overall sensitivity of 93.54% and a specificity of 84.2%. However, the Dot ELISA is advantageous in terms of shorter test duration and the option of storage of the dotted antigen at room temperature for longer periods. Also, the test requires minimal infrastructure and instrumentation unlike ELISA, where an ELISA reader and washer are required.

Dot ELISA has become increasingly popular for the diagnosis of many other parasitic diseases also, such as filariasis,[8] malaria,[9] and schistosomiasis,[10] besides cysticercosis. Dot ELISA has been used earlier for diagnosis of cysticercosis using antigens derived from cysticercus cellulose,[11-13] cysticercus longicollis,[14] and T. solium glycoproteins.[15] Liu et al., 1996, have modified the Dot ELISA by Dot-immunogold silver staining for diagnosis of cysticercosis.[16]

We have found the Dot ELISA using cysticercus fasciolaris to be 88% sensitive and 74% specific. Though the commercial ELISA is 92% sensitive and 84% specific, the performance of Dot ELISA is comparable to it; and the differences between the values of sensitivity and specificity of the two tests are not statistically significant. Mandal et al. have also compared the performance of Dot Blot to ELISA, for the detection of antibodies against antigens from larval T. solium in children aged 5 to 12 years. Both tests gave similar sensitivity, viz., 89%; while ELISA had better specificity, viz., 81%, than Dot Blot (73%).[17] In another study by Biswas et al., Dot-ELISA was found to be 56.25% sensitive and 92% specific, as compared to plate ELISA, which was 43.75% sensitive and 98% specific.[10] Jiang et al. have observed confirmed cases of neurocysticercosis showing 81.6% to 96.1% positive reactions in Dot-ELISA.[11] It is reported that sensitivity and specificity of Dot ELISA generally vary from 56.25% to 97.61% and from 90.6% to 92% respectively.[11,12,14] The difference can be explained by different criteria for patient selection and different gold standards used in different studies. We have used visibility of scolex in scans, an absolute diagnostic criterion, which also denotes active lesions; and hence seropositivity was high despite presence of a single cyst in several cases.

To detect any cross reactivity of intestinal parasites, a probable cause of false positives, the study group was subjected to stool test. We observed two cases with Ascaris and three cases with E. histolytica/E. dispers in stool. The sera of these cases did not cross react in Dot ELISA and commercial ELISA. No case of intestinal teniasis was encountered in the current study. Cross reactivity of the Cysticercus fasciolaris antigen has been evaluated in our earlier study evaluated in our earlier study, where we observed that the antigen does not cross react with cases of intestinal teniasis, hydatid disease, and Hymenolepis nana, which were included as diseased controls.[8] The high false positivity in serological testing is a general diagnostic fallacy in regions with high endemicity for cysticercosis due to persistence of antibodies following exposure. Further presence of cysts at other sites, including muscle and subcutaneous tissue, cannot be completely and convincingly ruled out in controls by clinical screening methods. These cysts can give an immuno-positive result in serological tests, which is then erroneously categorized as false positive.

We conclude that Dot ELISA is a simpler test with sensitivity comparable to ELISA. Cysticercus fasciolaris can be used as an alternative source of antigen in immunodiagnostic tests for the human pathogen. While the test may be used for screening for seropositivity in suspected cases, the localization and staging of the cyst can be done by radio-imaging techniques. The test needs to be evaluated as a screening procedure in population-based studies, where sensitivity and specificity may vary due to varying disease endemicity and presence of other parasitic infections.

**Acknowledgment**

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**References**


**Background:** Young children living with parents who smoke are exposed to unacceptable health hazards. **Aim:** To determine patterns of parental smoking, the level of parental awareness about hazards of secondhand smoke, and the effect of risk awareness on smoking behavior. **Setting:** Health centers affiliated with two teaching hospitals in Tehran. **Design:** Cross-sectional. **Materials and Methods:** Data was collected from parents of preschool children visiting the health centers, through face-to-face interview, during a period of 18 months. **Statistical Analysis:** Data was analyzed by multiple logistic regression, and analysis of variance was done for comparison of means. **Results:** In a total of 647 families, prevalence of parental smoking was 35.7% (231 families). In 97.8% of smoking families, only the fathers smoked; and in 5 (2.2%) families, both parents were regular smokers. Prevalence of smoking was higher in poor families as compared with families who were well-off (39% vs. 25%; P = 0.025), and also in families with lower educational level. There was no significant difference in risk awareness between smokers and nonsmokers (P > .05). **Conclusion:** Low socioeconomic status and low education were identified as risk factors for children's exposure to secondhand smoke; parental risk awareness had no apparent effect on the smoking behavior. Unlike western societies, fathers were the sole habitual smokers in most families. Since factors that influence smoking behavior vary in different cultures, interventional strategies that aim to protect children from the hazards of tobacco smoke need to target diverse issues in different ethnic backgrounds. **Key words:** Children, secondhand smoke, tobacco

**Introduction:**

Tobacco smoke is a complex mixture of more than 4000 chemical compounds, including 43 known carcinogens, and cigarette use is a leading preventable cause of death in industrialized countries.[1-4] Secondhand smoke (SHS) is a potentially preventable environmental pollutant linked with respiratory problems, and parental smoking has been associated with increased rates of sudden infant death syndrome, otitis media, asthma, and decreased lung growth.[5] There is no safe level of exposure to...