

A STUDY ON THE CULTURE MEDIUM ANTIGENS OF *CYSTICERCUS CELLULOSAE* FOR DETECTING ANTIBODIES OF CYSTICERCOSIS BY MEANS OF ABC-ELISA

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Abstract. Two antigens of *Cysticercus cellulosae*, cystic fluid antigen (CFA) and the culture medium antigen (CMA), were used in Avidin - Biotin Peroxidase Complex - ELISA (ABC - ELISA) to detect IgG antibodies in 45 cases of cysticercosis treated with praziquantel. The results revealed the total positive rates as 51.11% with CMA and 82.22% with CFA. The positive rates in the cases treated within 2 courses of treatment were 79.17% for CMA and 87.50% for CFA, and only 19.05% for CMA and 71.43% for CFA in the cases treated for more than 3 courses. The fact that the positive rates decreased as the courses of treatment increased showed that the sensitivity of CMA might be related to the vital conditions of the worms in the body, whether alive or dead. It is, therefore, recommended that CMA has the potential to be employed in ABC - ELISA both as an indicator for diagnosing cysticercosis and as a reference for the evaluation of the treatment.

INTRODUCTION

Studies on ELISA with antigens from the fluid of *Cysticercus cellulosae* were carried out for the immunodiagnosis of cysticercosis (Arwin *et al*, 1982; Flisser *et al*, 1979; Larralde, 1986). During the study on culture medium fluid of *Cysticercus cellulosae*, we obtained a group of proteins from the fluid which contained proteins different from the ones in the cysticerci fluid and bodies. In order to study the antigenic specificity of the culture medium proteins and their significance as an antigen in detecting the antibodies in the patients with cysticercosis by means of ABC - ELISA, this study employed two antigens of *Cysticercus cellulosae*, cysticerci fluid antigen (CFA) and the culture medium antigen (CMA) in ABC - ELISA to detect IgG antibodies in 45 patients with cysticercosis who had been treated with praziquantel in different courses (25 mg/kg body weight daily for 5-6 days, at 3 months intervals) The possibility of CMA employed in ABC - ELISA as an indicator for the evaluation of the treatment is presented in this report.

MATERIAL AND METHODS

Major reagents

The set of reagents for ABC - ELISA were products of Shanghai Bioproduct Institute.

Antigens

CFA extract was prepared from pork cysts. Cysts isolated pig skeletal muscle were washed in three changes (5 minutes, each) of 0.8% saline and the fluid was then extracted from the cysts and centrifuged for 20 minutes, at 3,500 rev/minutes. The supernatant fluid was stored at 4°C.

CMA was prepared as described by Andrews *et al*, (1979) for culturing in Ringer fluid with out any protein for 24 hours at 37°C and then the cysts were removed, and the medium fluid was centrifuged. The supernatant was stored at 4°C.

Sera

45 cases of cysticercosis were clinically con-

firmed and had been treated with praziquantel for 0-9 courses of treatment.

Positive control sera : 5 positive cases of the patients with IHA and their sera were pooled.

Negative control sera : 5 uninfected persons were found negative with IHA and their sera were pooled, 20 sera were also collected from healthy people.

Methods : ABC - ELISA

The steps were taken as described by Li (1986), concentration of antigens being 10 µg/ml and the sera samples were diluted to 1 : 400.

Evaluation

The OD value of the samples was evaluated at 492nm, the positive value being determined by a value as much as twice or more than of the negative control.

RESULTS

A comparison between antigens for determination of special antibodies in 45 patients with cysticercosis was shown by means of ABC - ELISA (Table 1)

Table 1

Two antigens detecting special antibodies in 45 patients of cysticercosis by ABC-ELISA.

Antigen	Cases tested	Positive cases	Positive rate (%)
CFA	45	37	82.22
CMA	45	23	51.11

There was a significant difference between two antigens (p<0.01). No false positive occurred in the 20 cases of uninfected sera for CMA, but 2 false positive cases were found for CFA.

The results of the 45 cases detected with the two antigens were related to courses of treatment. (Figs 1, 2)

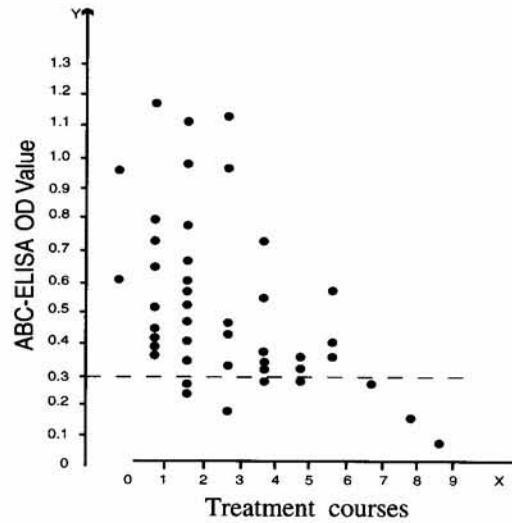


Fig 1 - Results with CFA for the patients treated with different course.
--- (above the line were positive).

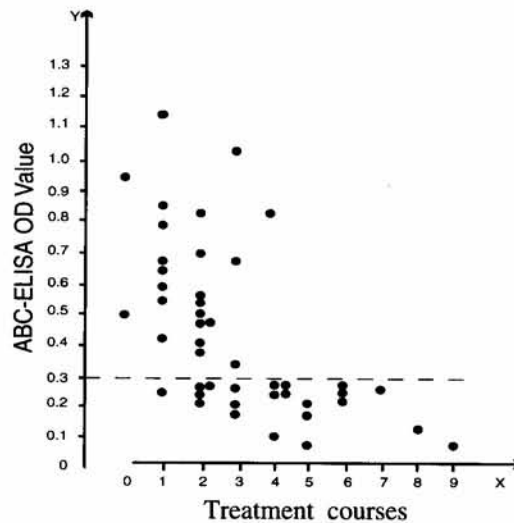


Fig 2 - Results with CMA for the patients treated with different courses.
---(above the line were positive).

The positive rates in the cases treated within 2 courses of treatment were 79.17% (19/24) for CMA and 87.50% (21/24) for CFA (p > 0.25), and were only 19.05% for CMA and 71.43% for CFA in the cases treated for more than 3 courses (p < 0.01). There was a significant difference between the positive rates of patients within 2 courses and more than 3 courses of

treatment with CMA but no significant difference of those were found with CFA.

The relation between the results with CMA and the infective conditions of patients and the curative effect is as follows.

Five cases treated within 2 courses were found negative with CMA, among whom 2 cases were negative with CFA. These patients belonged to the type of subcutaneous and intermuscular tissues infections. After 2-course treatment, the cysts in their subcutaneous tissues disappeared.

Three negative cases for CMA were also infected slightly.

Four cases treated for more than 3 courses were positive with CMA. They all belonged to the serious and mixed type of infection. For most of the patients treated for 3-9 courses, the CMA group was negative while the CFA was positive. The clinical symptoms of these patients improved greatly through the treatment.

DISCUSSION

Different results for the two antigens for the same subjects suggested that the specific properties of the two antigens were different. Antigens of parasites are quite complex but they can be divided into somatic and metabolic antigens on the basis of their origins. (Zhao, 1982). CMA was mainly from the excretion and secretions of living worms while CFA was the base of the somatic proteins (Arwin *et al*, 1982) which led to the differences.

According to the clinical reports, CT images of most patients with cerebral cysticercosis showed that the cysts began to subside or calcify and biopsy reports on the subcutaneous cysts indicated damaged cyst walls or killed worms after several courses of treatment. However, only a few cases had their symptoms and signs improved remarkably. Blindness

often occurred during the treatment. It is highly recommended that an effective method for the evaluation of the treatment be established. The excretory and secretory antigens of the worms in the body could vanish and their relevant antibodies could decrease during the courses of treatment. As a result, the results might be negative with CMA. On the other hand, the positive results with CFA might be due to the dead worms still existing and effecting the production of antibodies.

The results of the study suggested that the sensitivity of CMA might be related to the vital conditions of the worms in the body, whether alive or dead. It is, therefore, recommended that the potential of CMA be employed in ABC-ELISA both as an indicator for the diagnosis of cysticercosis and as a reference for the evaluation of the treatment.

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