EVALUATION OF COUNTERIMMUNOELECTROPHORESIS FOR SERODIAGNOSIS OF HUMAN CYSTICERCOSIS

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INTRODUCTION

Serology of cysticercosis is of interest as a means of supporting clinical diagnosis of human neurocysticercosis. Several methods have been evaluated; they are indirect haemagglutination test (IHA) and double diffusion in agar (Proctor *et al.*, 1966; Powell *et al.*, 1966; Rydzewski *et al.*, 1975; Botero and Castano, 1982; Loo and Braude, 1982; Mahajan *et al.*, 1982); immunoelectrophoresis (IEP) (Flisser *et al.*, 1980); enzyme-linked immunosorbent assay (ELISA) (Espinosa *et al.*, 1982; Diwan *et al.*, 1982); and radioimmuno assay (RIA) (Miller *et al.*,1984). Despite this extensive work, there is still a need for a new serological method which is sensitive, specific, rapid and simple.

Counterimmunoelectrophoresis (CIEP) is rapid, easy to perform, and has been applied to detect antibodies in bacterial, viral and parasite infections (Draper, 1976). The present investigation was therefore to evaluate CIEP in comparison to the widely used IHA in terms of its sensitivity and specificity for serodiagnosis of human cysticercosis. Furthermore, soluble *Taenia saginata* adult worm extract was also to be employed as an antigen is CIEP in addition to commonly used *Cysticercus cellulosae* extract and the results compared.

MATERIALS AND METHODS

Sera: A total of 11 confirmed cysticercosis sera were obtained from cysticercosis patients. Six of them had neurological symptoms and cysticerci removed either from the spinal cords or subcutaneous tissues. Four patients had neurological symptoms with the presence of subcutaneous cysts indicative of cysticercosis. The last serum was from a child with cysticercus removed from vitreous humour of an eye.

Blood donor sera were obtained from the Blood Bank Unit, Maharaj Nakorn Chiang Mai Hospital, Faculty of Medicine, Chiang Mai University, Chiang Mai.

Helminthiasis sera were collected from cases proved to be helminth-infected by formalin-ether concentration method of stool examination. Most of these sera were kindly provided by Mr. Uthai Thomyamongkol, Chaiyaphum Hospital, Chaiyaphum Province. The remainder were collected from cases at Maharaj Nakorn Chiang Mai Hospital. Of a total of 75 cases, 36 had *Opisthorchis*, 14 had *Opisthorchis* and hookworm, 10 had *Opisthorchis* and *Fasciola*, 8 had *Strongyloides*, 3 had hookworm, 1 had *Strongyloides* and hookworm, and 1 had *Capillaria philippinensis*. Two of the remaining cases had a mixed infection of 3 helminths.

Helminth-negative sera were collected from cases whose stools were negative for helminth eggs or larva by concentration method.

Suspected cysticercosis sera were obtained from patients with neurological symptoms and their sera were positive against *C. cellulosae* extract by CIEP.

Anti-cysticercus serum globulin produced in pig was kindly provided by Dr. Ana Flisser, Departmento de Immunologia, Instituto de Investigaciones, Biomedicas, Ciudad Universitaria 20, D.F., Mexico.

Antigens : *Cysticercus cellulosae* extract was prepared as described by Flisser *et al.*, (1980). Briefly, cysticerci proved to be larva of *Taenia solium* were isolated from infected pork. They were homogenized in 3 M KCl in 0.01 M phosphate buffer saline, pH 7.2 (3 M KCl-PBS) with the aid of tissue grinder. The homogenate was left overnight in the refrigerator with continuous stirring and subsequently centrifuged at 10,000 × g for 30 min. at 4°C. The supernatant fluid was extensively dialysed against PBS and its protein content was determined according to Lowry *et al.*, (1951). The extract was then aliquoted and lyophilized.

Taenia extract was prepared from *Taenia saginata* adult worms obtained from autopsy. Well-cleaned whole worm was homogenized in 3 M KCl-PBS with the aid of Waring blender. The homogenate was next ground with the tissue grinder and the milky suspension was left overnight in the refrigerator and continuously stirred. Following centrifugation at 10,000 × g for 30 min, the supernatant fluid was extensively dialysed against distilled water and any precipitate formed was removed by centrifugation. The extract was next aliquoted into 7 ml portions into centrifuge tubes and 3 ml diethyl ether was added. The tubes were stoppered, shaken vigourously, and centrifuged at 500 × g for 1 min. The bottom aqueous layer was transferred to another tube and the procedures repeated once more. The aqueous extract was finally removed, aliquoted and lyophilized. Protein determination was later made from reconstituted antigen powder.

Serological methods : Counterimmunoelectrophoresis was performed as described by Krupp (1976). Six ml of melted agar (Ion agar No. 2, Oxoid Limited, London) in 0.05 M Veronal buffer, pH 8.6 (VB) was poured onto a 2 × 3 inch agar-coated glass plate. After the agar solidified, pairs of wells were cut. Wells were 3 mm in diameter with the distance between antigen and serum wells 3 mm from the margin. Wells near the cathode were filled wih 10 µl of antigen which has the protein content of 8 mg/ml; and the opposite wells were filled with 10 µl of tested sera, which were previously concentrated twice by lyophilization and reconstitution. Tank buffer was VB of the same molarity. Electrophoresis was carried out at constant current at 10 mA per plate for 1 hr. Plates were next immersed in 5% sodium citrate to remove nonspecific precipitin bands (Dasgupta *et al.*, 1984), then in 0.85% NaCl for 4-8 hr. This was followed by drying agar at 45°C and stained with 0.5% Amido black (Crowle, 1973). The positive reaction was indicated by precipitin band (s) formed between antigen and serum wells.

The indirect haemagglutination test was carried out as described by Morakote *et al.*, (1984). The antigen used for coating tanned human O cells was *C. cellulosae* extract. A titer of 1:512 or more was considered as positive.

RESULTS

To compare sensitivity and specificity of CIEP to those of IHA, several groups of sera were examined by both methods using *Cysticercus cellulosae* extract as antigen and the results are summarized in Table 1. It can be seen that CIEP detects antibodies in 7 of 11 (sensitivity 64%) and IHA detects 6 of 11 confirmed cysticercosis sera (sensitivity 55%). Among these 11 sera, 5 were positive by both CIEP and IHA, 5 were positive by either CIEP of IHA, and 1 was negative by both methods. When tested with 130 control sera including blood donors, helminthiasis, and *Taeniasis saginata*, only 2 of them were

Table 1

Sensitivity of counterimmunoelectrophoresis (CIEP) and indirect haemagglutination test for diagnosis of cysticercosis.

Group	Total No. examined	No. positive	
		CIEP	IHA
Confirmed cysticercosis	11	7	6
Blood donors	49	0	0
Helminthiases	75	1	2
Taeniasis saginata	6	1	0

Table 2

Comparison of *Taenia saginata* adult worm and *Cysticercus cellulosae* extract by CIEP for diagnosis of human cysticercosis.

Group	Total No. examined	No. positive	
		Cysticercus antigen	Taenia antigen
Cysticercosis*	16	14	12
Blood donors	18	0	0
Helminthiases	75	1	0
Helminth-negative	32	1	0
Taeniasis saginata	9	1	0

* Confirmed and suspected cysticercosis.

positive by each method, demonstrating high specificity. It was thus concluded from the results that CIEP has sensitivity and specificity close to those of IHA.

The use of *T. saginata* adult worm antigen produced greater sensitivity than the use of *C. cellulosae* in serodiagnosis of human cysticercosis was reported by Rydzewski *et al.*, (1975). Thus, *Taenia saginata* worm extract and *C. cellulosae* extract were tested by CIEP with several groups of sera, and the results are presented in Table 2 and Fig. 1. While 14 of 16 cysticercosis sera reacted with *C. cellulosae* extract, only 12 showed reaction against *T. saginata* extract. Nonetheless, none of the control sera, i.e., helminthiases, blood donors, and stool-negative sera, was positive with

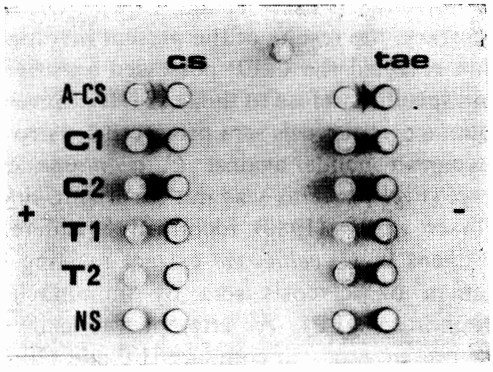

Fig. 1—Showing precipitin bands in counterimmunoelectrophoresis after staining with Amido black. Abbreviations: A-CS, pig gamma globulin anti-cysticercus; C1 & C2, confirmed cysticercosis serum case 1 & 2; T1 & T2 *Taeniasis saginata* serum case 1 & 2; NS, Normal human serum; cs, *Cysticercus cellulosae* extract; tae, *Taenia saginata* adult worm extract.

Taenia extract. On the contrary, 3 were positive with cysticercus extract. Most of cysticercosis sera produced clearly visible precipitin bands against both *Taenia* and cysticercus extract (Fig. 1). From these results it appears that *Taenia* antigen can be used in CIEP for serodiagnosis of cysticercosis with only little loss of sensitivity, but higher specificity.

DISCUSSION

Serodiagnosis of cysticercosis is not new but it should be carefully evaluated, especially if the population under study lives in geographically different areas. This holds true among Thai communities where multiple helminthic infections are common, and which may create false positivity in serological tests so employed. Moreover, a rapid, easily performed and interpreted serological test is more welcome than time-consuming and complicated tests. The counterimmunoelectrophoresis (CIEP) method was thus evaluated in the present study.

In the past, CIEP was found to be an insensitive method for serodiagnosis of human cysticercosis (Flisser *et al.*, 1979). On the contrary, the results of the present investigation revealed the CIEP possessed sensitivity and specificity close to those of IHA. Several human cysticercosis sera produced 1-4 strong precipitin bands against *C. cellulosae* extract (Fig. 1). This was not surprising since Flisser *et al.*, (1980) identified 8 different antigens in *C. cellulosae* extract reacting to human cysticercosis sera by immunoelectrophoresis (IEP). An attempt was made in the present study to compare IEP and CIEP, and the result was unsatisfactory due to the weak IEP reaction. Probably the patients under this study have weaker antibody response than Mexican patients. CIEP was recently applied for detection of porcine cysticercosis (Pathak *et al.*, 1984).

Crude *Taenia saginata* adult worm extract could be employed in CIEP with little loss of sensitivity. This was in agreement with Rydzewski *et al.*, (1975) who showed by IHA and agar gel precipitin test that the numbers of human cysticercosis sera reacting with *T. saginata* antigen were the same as those reacting with *C.cellulosae* antigen. The ability to use *T. saginata* extract in CIEP demonstrated the presence of common antigens. This was supported by the results of Flisser *et al.*,(1982) who found that anti-cysticercus serum produced 5 precipitin bands against *T. saginata* extract. In addition, Rydzewski *et al.*, (1975) showed that human cysticercosis sera reacted to both *C. cellulosae* and *T. saginata* adult worm antigens by IHA, immunofluorescence, and agar precipitin test. It thus appears from the above results that *T. saginata* adult worm extract can be used in CIEP when cysticerci of *T. solium* are not available.

The sensitivity of IHA found in the present study fell in the range reviewed by Flisser *et al.*, (1979), i.e., 10-92%. Although the sensitivity of IHA can be improved by lowering the significant titer to 1:128 or less, this will create unwanted higher false positive rate. Mahajan *et al.*, (1982), and Procter *et al.*,(1966) were able to get 86% and 85% sensitivity respectively while Powell *et al.*, (1966) got 82%, Rydzewski *et al.*, (1975) - 75% and Botero and Castano (1982)-62%. This wide range of sensitivity could be due to different antigen preparations (Rydzewski *et al.*, 1975), different sites of cysticerci in patient's brain (Miller *et al.*, 1984), or poor immune response of the patients (Flisser *et al.*, 1980). IHA was suggested to be the best test in serodiagnosis of cysticercosis (Loo and Braude, 1982).

In view of the fact that many human cysticercosis sera were positive by CIEP, but less by IHA, performing both tests with a single serum will increase diagnostic sensitivity for human cysticercosis.

SUMMARY

Counterimmunoelectrophoresis (CIEP) was evaluated in comparison to the indirect haemagglutination test (IHA) for serodiagnosis of human cysticercosis. It was found that CIEP detected antibodies in 7 of 11 (64%) and IHA detected them in 6 of 11 (55%) confirmed cysticercosis sera. Only 2 of 130 control sera were positive by each technique. *Taenia saginata* adult worm extract was found to be satisfactory for use in CIEP in place of *Taenia solium* cysticercus extract, with only little loss of sensitivity. Finally, CIEP in combination with IHA greatly increased the diagnostic sensitivity for human cysticercosis.

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