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SPINAL FLUID IMMUNOENZYMATIC ASSAY (ELISA) FOR NEUROCYSTICERCOSIS

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SUMMARY

Three different antigens from **Cysticercus cellulosae**, vesicular fluid, a saline and an alkaline extract, were studied in the immunoenzymatic assay (ELISA) for the diagnosis of cysticercosis. ELISA was then compared to immunofluorescence, complement fixation and hemagglutination tests in spinal fluids, for the diagnosis of neurocysticercosis. Results indicate the immunoenzymatic assay as a convenient test for this purpose.

INTRODUCTION

Cysticercosis is a serious public health problem in Brazil [4,7,12,22]. Among the several clinical forms (neurologic, ocular, muscular, subcutaneous) neurocysticercosis is certainly the most serious one. However, many cases have been diagnosed only by chance during neurosurgery or necropsis, or through spinal fluid examen performed for other purposes in non-suspected cases. This suggests the occurrence of a symptomless parasitism of large epidemiological significance. Thus the development not only of practical laboratory tests for routine purposes, but also of more reliable ones should be fundamental for determining the real prevalence of such parasitism [16].

For the serological diagnosis of cysticercosis the complement fixation test (CF) [23] shows a low sensitivity as well as a limited specificity due to cross-reactions observed mainly in other parasitic infections. Passive hemagglutination tests (HA) [8,15,17,19,20,21] although more sensitive, have shown a reduced specificity. Immunofluorescence tests (IF) with either fragments [14] or cryostat sections [9] of cysticercus, in spite of showing a significant percentage of non specific results have recently found frequent application for assaying spinal fluids or sera [2,3,13].

The immunoenzymatic assay (ELISA) for cysticercosis was described in 1978 by ARAMBULO et al. [1], using as antigens delipidized saline extracts from **Taenia solium** adult worm and from cysticerci. In the study of suspected cases of cysticercosis, this test has revealed a better sensitivity and specificity than the IF and HA tests.

To standardize the immunoenzymatic assay for cysticercosis, we have studied 3 different antigens from **Cysticercus cellulosae.** The test was then compared to CF, IF and HA tests in spinal fluids of patients with neurocysticercosis or other neurologic pathologies.

MATERIAL AND METHODS

Antigens

Cysticerci were carefully collected from infected pork. Vesicular fluid was pooled, centrifuged at 4,800 x g for 15 minutes at 4°C and supernatant stored at -70°C in small aliquots for use. A saline extract from about 50 cysticerci was prepared by grinding the parasites, previously emptied from vesicular fluid, in about 5 ml distilled water in a Potter-Eveljn tissue homogeni-

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zer, at 4°C for 3 minutes, and after adding 5 ml of 1.7% NaCl solution the mixture was kept at 4°C for 2 hours under slow agitation. After centrifugation at 10,000 x g for 30 minutes at 4°C, the supernatant was stored in small aliquots at -70°C. An alkaline extract was prepared in the same way by substituting a NaOH 0.3 N solution for the saline solution.

Antiglobulin conjugate

The IgG fraction of a sheep anti human IgG antiserum, prepared at the laboratory, was labeled with horseradish peroxidase (Type VI, Sigma Chem. Co., USA) by the technique of WILSON & NAKANE [24].

Immunoenzymatic assay

Wells in polystirene plates (Plásticos Ampla, São Paulo, Brazil) were filled with 200 microliters antigen solution in carbonate buffer 0.06 M, pH 9.6, and plates sensitized for 18 hours at 4°C. They were then washed 3 times with saline-phosphate buffer (NaCl 0.15 M, phosphates 0.01 M, pH 7.2) with 0.05% Tween-20 (PBS-Tween 20).

For the assays, plates were incubated with 200 microliters of spinal fluids or serum dilutions in PBS-Tween 20, washed, and incubated with 200 microliters conjugate diluted in PBS-Tween 20 according to titer. Incubations were done at 37°C for 45 minutes. Two hundred microliters substrate of 5.23 mM 5-aminosalycilic acid and 1.47 mM hydrogen peroxide were added to the wells plates incubated for 60 minutes at room temperature and the reaction stopped with one drop NaOH 1N. Tests were read at 450 nm in a Beckman DU-2 spectrophotometer.

Spinal fluids

Spinal fluids were obtained from 80 patients, 22 with proven neurocysticercosis, 21 clinically suspect of neurocysticercosis, 22 with epilepsy, 5 with neurosyphilis and 10 individuals with the only complain of cephalea. Spinal fluids were kept in aliquots at -20°C in siliconized tubes, after centrifuged for removing any solids.

Other tests

CF tests were performed by Kolmer's technique with an alcoholic extract of **Cysticercus cellulosae** as antigen [11]. For the HA test, aldehyde-fixed human red cells were sensitized with vesicular fluid of cysticercus and tests done as described [5,6]. IF tests were performed with cysticercus fragments fixed on microscope slides [14].

RESULTS

Proteins and polysaccharides in antigens

Proteins were determined by the biuret [10], and polysaccharides by the antrona test [18]. Table I shows results obtained for the 3 cysticercus antigens.

TABLE I

Proteins and polysaccharides in different **Cysticercus cellulosae** antigens

Antigens	Proteins	Polysaccharides
Vesicular fluid	8.0 mg/ml	5.0 mg/ml
Saline extract	5.5 mg/ml	3.5 mg/ml
Alkaline extract	4.0 mg/ml	2.0 mg/ml

Evaluation of the different parasite antigens in the immunoenzymatic assay

Plates were sensitized with antigen dilutions from 10 μg to 40 μg proteins per milliliter and assays performed with a reactive serum from a patient with cysticercosis and a non-reactive serum from a normal case. For the three antigens the 20 μg/ml dilution furnished maximal reactivity, and was thus selected for assays. Threshold for positive results was taken as 5 0 ELISA Units (1 E.U. = Optical Density x 100). Spinal fluid titers were given as the highest dilution presenting a positive result.

Figure 1 shows reactivities found for the positive and the negative standard sera with the different antigens at 20 μg/ml.

Immunoenzymatic assay of the spinal fluids

Spinal fluids were assayed undiluted or in doubling dilutions in plates sensitized with the saline extract antigen. This antigen was chosen because giving reactivities of less than 1.0 E.U. for non diluted spinal fluids of normal individuals or patients with no cysticercosis.

As shown in Table II, ELISA was positive for all the 22 patients with neurocysticercosis, while IF was positive in 20 patients, FC in 19

and HA in 17. ELISA titers in this group ranged from 2 to 512, with 24.1 as the geometric mean titer. The cases presenting all 4 test positive, which corresponded to 63.6% of this group, showed an ELISA geometric mean titer of 47.6, while giving a g.m.t. of 11.3 for the cases with 3 positive tests (27.2%), and a g.m.t. of 2.0 for the cases with only 2 positive tests (9.1%).

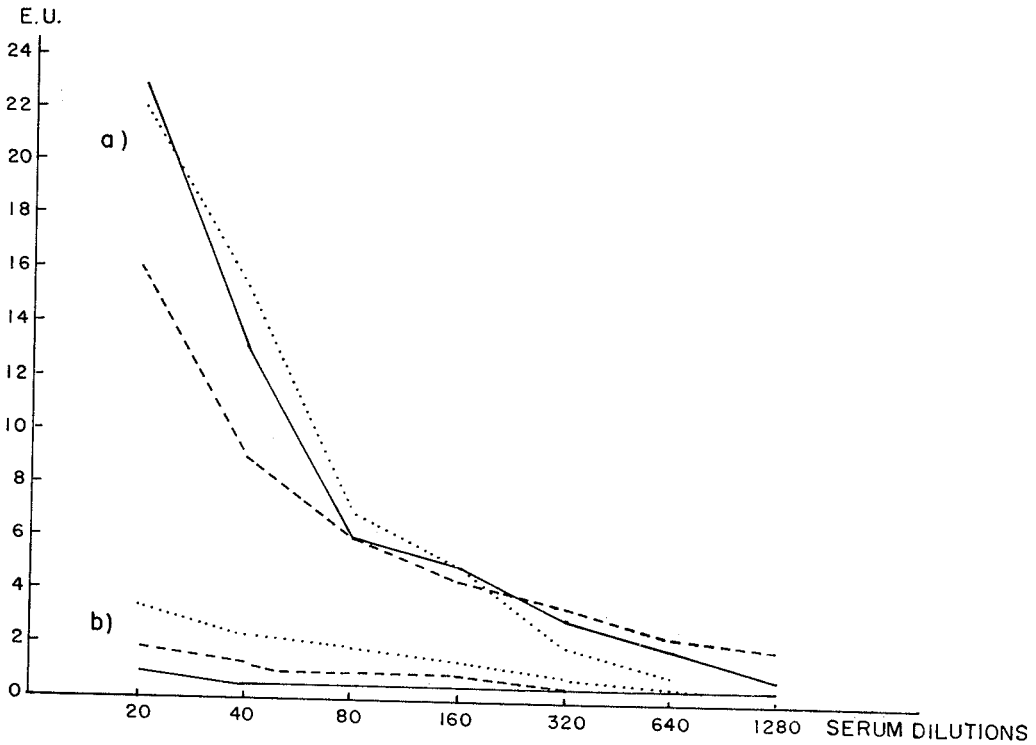

Fig. 1 - ELISA reactivity curves of a positive (a) and a negative (b) serum with vesicular fluid (—) saline-extract (·····) and alkaline-extract (----) of *Cysticercus cellulosae* (E.U. = Optical Density x 100).

TABLE II

Frequency of agreement between spinal fluid tests for neurocysticercosis

Serologic pattern				Diagnosis				
FC	HA	IF	EL	Neurocysticercosis	Clinically suspect cases of neurocysticercosis	Epilepsy	Neurosyphilis	Cephalea
+	+	+	+	14 (63.6%)	0	0	0	0
+	—	+	+	3 (13.6%)	0	0	0	0
—	+	+	+	3 (13.6%)	0	1 (4.5%)	0	0
+	+	+	—	0	1 (4.8%)	0	0	0
+	—	—	+	2 (9,1%)	0	0	0	0
—	—	+	+	0	1 (4.8%)	0	0	0
—	+	—	—	0	1 (4.8%)	0	0	0
—	—	—	+	0	1 (4.8%)	0	0	0
—	—	—	—	0	17 (81.0%)	21 (95.5%)	5 (100%)	10 (100%)
Totals				22	21	22	5	10

From the 21 clinically suspect cases of neurocysticercosis, only 2 showed a positive ELISA, with a mean titer of 2.0. However, ELISA was negative in one case with all 3 other tests positive.

One patient with epilepsy had a positive ELISA with a titer of 4.0 and IF and HA tests also positive.

All tests were negative for patients with neurosyphilis or cephalea.

DISCUSSION

When comparing different antigenic preparations from **Cysticercus cellulosae** we observed that both the vesicular fluid and extracts of the parasite gave satisfactory results in the immunoassay for cysticercosis. Obtaining vesicular fluid in sufficient amounts for routine work presents some practical problems. Extracts yielded larger amounts of antigen and thus were found as more convenient for antigen production. No significant antigenic differences were seen between the saline and the alkaline extract.

ELISA with the saline extract of **Cysticercus cellulosae** as antigen seems to be a rather sensitive and specific test for assaying spinal fluids for neurocysticercosis.

As an easily standardized and practical test for routine work, ELISA for cysticercosis should be further studied, mainly in relation to antigenic fractions of the parasite extracts, which could furnish even more sensitive and specific results.

RESUMO

Teste imunoenzimático (ELISA) para diagnóstico liquórico na neurocisticercose

Investigou-se o comportamento de três antígenos de **Cysticercus cellulosae**, o líquido vesicular, um extrato salino e um extrato alcalino, no teste imunoenzimático (ELISA) para a cisticercose.

Em seguida, para o diagnóstico da neurocisticercose, comparou-se esse teste com os testes de imunofluorescência, fixação do complemento e hemaglutinação, em amostras de líquido cefalorraqueano. Os resultados obtidos indicam o teste imunoenzimático como satisfatório para fins de rotina, em vista da sensibilidade, especificidade e facilidade de execução.

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