

**PASSIVE HAEMAGGLUTINATION TEST FOR HUMAN NEUROCYSTICERCOSIS
IMMUNODIAGNOSIS. II — COMPARISON OF TWO STANDARDIZED PROCEDURES FOR THE
PASSIVE HAEMAGGLUTINATION REAGENT IN THE DETECTION OF ANTI-*Cysticercus*
cellulosae ANTIBODIES IN CEREBROSPINAL FLUIDS**

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S U M M A R Y

A comparison of two different standardized reagent procedures for the passive haemagglutination test (PHA) in the detection of specific antibody to *Cysticercus cellulosae* in cerebrospinal fluid (CSF) was carried out. The formaldehyde-treated group O Rh-human red blood cells (HuRBC) and glutaraldehyde-treated sheep red blood cells (SRBC) were the supplies for the reagents preparation and, in the tests, they were designated as PHA-1 and PHA-2, respectively. For both reagents the cells were coated with the cysticerci total saline extract (TS) antigen. PHA-1 and PHA-2 were assessed in a total of 204 CSF from patients with neurocysticercosis, from non-related infections and from healthy individuals. The positivity and specificity indices obtained were respectively 81.7% and 94.4% for PHA-1 and for PHA-2, 88.7% and 96.6%. Since no significant differences were observed between the results provided by two reagents, at level of significance of 0.05, either processes of cell sensitization can alternatively be used according to the own laboratory convenience.

KEY WORDS: Neurocysticercosis; Passive haemagglutination test; Cerebrospinal fluid.

I N T R O D U C T I O N

Cysticercosis constitutes an important medical-sanitary and veterinary problem, particularly in tropical and subtropical areas of world, as a consequence of poor environmental sanitation. The disease arises from tissues and organs infections with *Taenia solium* larvae, *Cysticercus cellulosae*, and some individuals remain as asymptomatic for long period of time.

The neurocysticercosis, with the larvae lodged in the central nervous system (CNS), repre-

sents the most frequent and serious form of the disease. The parasite may produce clinical symptoms varying from headache, mental disturbances, mental and/or behaviour changes to convulsion crises, blindness, meningitis and meningoencephalitis¹⁰.

Neurocysticercosis diagnosis is usually made based upon cysticercus localization in CNS and by radiological demonstration of intracranial calcified cysticerci and epidemiological da-

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ta, although the cerebrospinal fluid analysis is also an useful diagnostic method.

Since an increase in cell number, usually of lympho-mononuclear cells and eosinophils, occurs in neurocysticercosis, the CSF presents change in the protein profile, owing to gammaglobulin arising, which is a relevant indications, mainly when immunological reactions display the presence of specific antibodies⁵.

The specific antibodies detection by means of practical, economic, sensitive and specific technique is the valuable diagnostic tool for several parasitic infections, including neurocysticercosis, even before the establishment of clinical symptoms.

The passive haemagglutination test (PHA) for neurocysticercosis diagnosis in CSF samples has been employed and shown as more sensitive than complement fixation test, and giving good correlation with indirect immunofluorescence test^{5, 6, 11}.

Depending on antigenic system, the process of sensitizing red blood cells was verified to influence greatly the sensitivity of PHA test results³⁴. This aspect still has to be studied in the diagnosis of neurocysticercosis.

Thus, the present investigation refers to our experience with the preparation of reagents for PHA test by two methodology and the evaluation of these as to their efficiency for the neurocysticercosis immunodiagnosis, not previously investigated.

MATERIALS AND METHODS

ANTIGEN: lyophilized saline total antigenic extract (TS) obtained from *Cysticercus cellulosae* was prepared according to the procedure previously described¹¹.

CEREBROSPINAL FLUID (CSF): one hundred-fifteen CSF samples collected from patients with proved neurocysticercosis confirmed by clinical, epidemiological and chemocytological data and some cases through computerized axial tomography; 89 CSF samples constituted the control group, of these 57 derived from pa-

tients with neurological clinical features other than cysticercosis and 32 samples obtained from presumably healthy individuals.

PASSIVE HAEMAGGLUTINATION TEST (PHA): two different methods (PHA-1 and PHA-2) of PHA test were standardized and *C. cellulosae* total saline antigen (TS) was employed for coating group O, Rh-, human red blood cells (PHA-1) and sheep red blood cells (PHA-2). For PHA-1 method, formalized human red blood cells (HuRBC) were coated with TS antigen, according to the procedure previously described by HOSHINO-SHIMIZU et al.^{3, 11}. For PHA-2 procedure, sheep red blood cells (SRBC) were sensitized with TS antigen as described by IMAI et al.⁴ with slight modifications. Briefly, to a 5% suspension of SRBC was added one to fourth of volume of 2.5% (v/v) glutaraldehyde (Merck, Brasil) diluted in 0,15M pH 7.2 phosphate buffered saline (PBS). After incubation for two hours at room temperature under gentle stirring, the cells were washed, resuspended to 5% in PBS containing 0.1% sodium azide (Merck, Brasil) and stored at 4°C. For use, a 5% cells suspension was washed and after last centrifugation, resuspended to the original volume and an equal volume of PBS containing 2.5 mg% of tannic acid (Hoeschst, Brasil) was added, followed by incubation for 10 minutes at 37°C. After tanning, the cells were washed and resuspended to original volume. An equal volume of TS antigen, at the optimal dilution in PBS, was added to the cell suspension and incubated for two hours at 37°C. The coated cells were washed and resuspended to 5% in stabilizing solution (pH 7.2 PBS containing 1% sucrose, 1% normal rabbit serum and 0.2% sodium azide). For use, to this cell suspension was added nine volumes of stabilizing solution to obtain 0.5% cell concentration. Following the same procedure the control cells (uncoated cells) were prepared.

For the assay, the polystyrene V-shaped 96-well microtitration plates (Inlab, Brasil) were employed. CSF sample was diluted in twofold series in pH 6.4 PBS for PHA-1 and in 7.2 PBS containing 0.1% bovine albumin (Sigma, USA) for PHA-2, starting from 1:1 dilution. Two series of 25 microlitres volume of each CSF dilution were prepared. Twenty-five microlitres of uncoated cells were added to one of the CSF dilution series

and 25 microlitres of coated cells to the second dilution serie. The plates were shaken (Titertek Plate Shaker, Flow Lab., USA) for 30 seconds and left to settle for 30 minutes for procedure PHA-1 and two hours, for PHA-2, at room temperature.

STATISTICAL ANALYSIS: the geometric mean titre (GMT) was calculated according to PAUL & WHITE⁷, expressing the titres based on the form $\log_2 (2^{n/2})$. The sensitivity, specificity, positive, negative predictive, as well as, the test efficiency indices were determined according to GALEN & GAMBINO². McNemar test was employed for analysing the agreement bet-

ween the results given by two PHA reagents in related CSF samples⁹.

RESULTS

The TS antigen protein¹ and polysaccharide⁸ contents were 6.1 mg/ml and 5.2 mg/ml, respectively, when 200 cysticerci in 20 ml final volume were employed for antigen preparation.

Table 1 shows the results obtained in two PHA procedures (PHA-1 and PHA-2) assaying CSF samples derived from patients with neurocysticercosis and from control group individuals.

TABLE I

Distribution of CSF antibody titres from patients with neurocysticercosis (a) and control group individuals (b), detected in PHA-1 and PHA-2 procedures.

TEST	TITRES GROUP	NR*	1	2	4	8	16	32	64	128	256	512	1024	2048	4096	8192	TOTAL
PHA-1	a	21	8	22	15	22	3	13	5	4	1	-	-	1	-	-	115
	b	84	3	2	-	-	-	-	-	-	-	-	-	-	-	-	89
PHA-2	a	13	-	11	10	13	12	11	13	11	6	3	4	5	2	1	115
	b	86	-	2	1	-	-	-	-	-	-	-	-	-	-	-	89

* NR = nonreactive

The sensitivity and specificity rates with confidence limit of 95% probability and GMT displayed by PHA-1 and PHA-2 tests are shown in Table 2.

Table 3 presents the results of positive and negative predictive values and PHA-1 and PHA-2 efficiency indices, which were estimated for different neurocysticercosis prevalence, that is, 100, 1,000 and 10,000 cases per 100,000 inhabitants or 0.1%, 1% and 10%, respectively.

The results obtained by two methodology were compared as to CSF reactivity and non-reactivity, and 89,2% agreement of results was observed. The co-positivity and co-negativity between the PHA-1 and PHA-2 procedures were 91.1% and 86.7%, respectively.

The analysis of the two PHA procedures agreement is presented in table 4.

DISCUSSION

The two standardized different procedures, PHA-1 and PHA-2, for anti-*Cysticercus cellulosae* antibodies detection in CSF samples were evaluated.

For coating either HuRBC or SRBC, employed for PHA-1 and PHA-2 procedures, respectively, it was employed the cysticerci total saline extract (TS) antigen kept lyophilized.

One hundred-fifteen CSF samples from patients with proved neurocysticercosis presented positivity of 81.7% and 88.7%, respectively for PHA-1 and PHA-2 procedures, and the antibody

TABLE II

Sensitivity and specificity rates with confidence limits of 95% probability (CL_{95%}) and geometric mean titres (GMT) of CSF from patients with neurocysticercosis (a) and control group (b) analysed by means of PHA-1 and PHA-2 procedures.

TEST	SENSITIVITY		SPECIFICITY		GMT
	$\frac{\text{POSITIVE}}{\text{TOTAL}}$	% (CL 95 %)	$\frac{\text{NEGATIVE}}{\text{TOTAL}}$	% (CL 95 %)	
PHA - 1	$\frac{94}{115}$	81.7 (74.5 - 88.9)	$\frac{84}{89}$	94.4 (89.6 - 99.2)	a - 4.5 b - 0.5
PHA - 2	$\frac{102}{115}$	88.7 (83.2 - 94.4)	$\frac{86}{89}$	96.6 (93.1 - 100)	a - 46.5 b - 0.5

TABLE III

Positive and negative predictive values and efficiency indices of PHA-1 and PHA-2 tests, according to neurocysticercosis prevalence.

TEST	POSITIVE PREDICTIVE VALUE (%)			NEGATIVE PREDICTIVE VALUE (%)			TEST EFFICIENCY (%)			
	PREVALENCE (%)	0.1	1	10	0.1	1	10	0.1	1	10
PHA - 1		1.4	12.9	61.9	99.9	99.8	97.9	94.4	94.3	93.2
PHA - 2		2.7	21.9	75.6	100	99.9	98.8	96.8	96.7	96.0

TABLE IV

McNemar χ^2 test for sensibility analysis between the procedures 1 and 2 employed for passive haemagglutination test.

TEST	PHA - 1			TOTAL
	RESULT	+	-	
PHA - 2	+	92	13	105
	-	7	92	99
TOTAL		99	105	204

titres ranging from 1:1 to 1:2,048, and from 1:2 to 1:8,192 (Table 1). These findings indicate high reactivities of PHA tests, when compared with the results reported by MARTINEZ-CAIRO et al.⁶, who found 68% sensitivity. To determine the specificity, 89 CSF samples from cases with no neurocysticercosis were assayed. Among these, five were positive for PHA-1 and two of them being derived from patients with viral meningitis (titre 1:2), one from patient with hydrocephalus (titre 1:1) and two from presumably healthy individuals (titre 1:1). In the PHA-2, three CSF samples were positive; two of them derived from patients with undetermined bacterial meningitis (titres 1:2 and 1:4) and one from presumably healthy individual (titre 1:2). Therefore, the specificity indices resulted in this study were 94.4% and 96.6% for PHA-1 and PHA-2, respectively.

The data were analysed based on low prevalence of disease in our population, providing therefore low positive predictive values (Table 3).

No significant differences were seen between the results furnished by PHA-1 and PHA-2, at level of significance of 0.05 ($\chi^2_{\text{obs.}} = 1.80$; $\chi^2_{1; 5\%} = 3.84$).

The PHA test, owing to its relative sensitivity, low cost, quickness in performing, as well as, easy preparation process of reagent, indica-

tes to be an efficient tool and adequate to be employed as laboratorial support for neurocysticercosis diagnosis by CSF samples.

This study suggests that to achieve the test's efficiency for neurocysticercosis immunodiagnosis, an accurate and suitable antigen preparation seems to be one of the essential requirements rather than the methodology employed for coating different red cell species.

RESUMO

Reação de hemaglutinação passiva para o imunodiagnóstico da neurocisticercose humana. II — Comparação de duas metodologias para o preparo de reagente para a reação de hemaglutinação passiva na detecção de anticorpos anti-*Cysticercus cellulosae* no líquido cefalorraquiano.

São descritas duas metodologias para o preparo de reagente para a reação de hemaglutinação passiva (RHA) destinada à pesquisa de anticorpos anti-*Cysticercus cellulosae* no líquido cefalorraquiano (LCR). Foram utilizadas hemácias humanas O Rh-formolizadas (HA-1) e hemácias de carneiro tratadas com glutaraldeído (HA-2), sensibilizadas com extrato antigênico salino total (ST) de cisticercos. Cento e quinze amostras de LCR de pacientes com neurocisticercose foram ensaiadas pelos testes HA-1 e HA-2, resultando em positividade de 81,7% e 88,7%, respectivamente. A especificidade obtida foi de 94,4% para HA-1 e 96,6% para HA-2. Não foi observada diferença significativa quanto à capacidade diagnóstica dos reagentes na RHA em neurocisticercose ($p < 0.05$).

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