PASSIVE HAEMAGGLUTINATION TEST FOR HUMAN NEUROCYSTICERCOSIS IMMUNODIAGNOSIS. I. STANDARDIZATION AND EVALUATION OF THE PASSIVE HAEMAGGLUTINATION TEST FOR THE DETECTION OF ANTI-Cysticercus cellulosae ANTIBODIES.

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SUMMARY

A passive haemagglutination test (PHA) for human neurocysticercosis was standardized and evaluated for the detection of specific antibodies to *Cysticercus cellulosae* in cerebrospinal fluid (CSF). For the assay, formaldehyde-treated group O Rh human red cells coated with the cysticerci crude total saline extract (TS) antigen were employed. A total of 115 CSF samples from patients with neurocysticercosis was analysed, of these 94 presented reactivity, corresponding to 81.7% sensitivity, in which confidence limit of 95% probability (CL95%) ranged from 74.5% to 88.9%. Eighty-nine CSF samples derived from individuals of control group presented as nonreactive in 94.4% (CL95% from 89.6% to 99.2%). The positive and negative predictive values were 1.4% and 99.9%, respectively, considering the mean rate of that this assay provide a rapid, highly reproducible, and moderately sensitive mean of detecting specific antibodies in CSF samples.

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

INTRODUCTION

Human cysticercosis, a disease caused by *Cysticercus cellulosae*, the *Taenia solium* larva, is a serious public health problem, mainly in developing countries. The *Cysticercus cellulosae* may situate in some human body structures or organs, however the most serious and frequent form is central nervous system localization.

A heterogeneity of clinical manifestations occurs in neurocysticercosis. These may be asymptomatic or symptomatic including convolution accompanied by intracranial hypertension or not and, occasionally, it is necessary to remove the parasite by surgery. The laboratorial diagnosis assaying cerebrospinal fluid (CSF) for specific antibody detection, may institute adequate treatment and thus, prevent progression to serious forms.

A passive haemagglutination test (PHA) for *Cysticercus cellulosae* antibody for neurocysticercosis diagnosis has been employed by several...
authors\textsuperscript{1, 8, 10, 12, 13}, using different antigens. BIA GI et al.\textsuperscript{1} described a PHA test employing aceto­ne treated and phosphate buffer saline (PBS) extracted cysticerci antigen. PROCTOR et al.\textsuperscript{13} sensitized erythrocytes with delipidized cysti­cerci saline extract antigen. POWELL et al.\textsuperscript{12} used as antigen the total crude extract from Cys­ticercus cellulosae. MARTINEZ CAIRO et al.\textsuperscript{8} described a PHA test utilizing erythrocytes with membrane and scolex antigens from cysticerci, and considering that the low concentration of immunoglobulin in CSF should be a limitation for detecting specific antibodies, they employed ammonium sulphate five-fold concentrated sam­ples. NASCIMENTO & MAYRINK\textsuperscript{10} evaluated the PHA test in sera, using different antigens extracted from cysticerci. These authors obser­ved that the scolex antigen was more specific than total crude antigen and those extrated from cysticerci vesicular fluid and membrane. More recently, COSTA\textsuperscript{3} reported a 100% positivity with cysticerci total saline extract antigen, in enzyme immunoassay (ELISA).

As this antigen has not been tried before in any other serological tests, further assessment of this Cysticercus cellulosae total saline ex­tract antigen for detecting antibody in CSF from patients with neurocysticercosis was performed by PHA test.

MATERIAL AND METHODS

ANTIGEN — Cysticerci obtained from natur­ally infected pig muscle and viscera were ex­haustively washed in saline solution (0.15M NaCl). The crude total saline extract antigen (TS) was obtained as described by COSTA (1983)\textsuperscript{3}, with some modifications. Briefly, about 200 cysticerci were homogenized in 10 ml of distil­led water in tissue grinder Potter (Scientific Glass Apparatus Inc. USA), and disrupted by sonication (Thornton-Inpec Eletrônica. Brasil) at four periods of 60 seconds — 20Khz — 1mA, in an ice bath. After adding 10 ml of 0.3M NaCl solution for isotonization, the mixture was soni­cated again and left overnight at 4°C with gentle stirring. After centrifugation at 6,500 x g for 30 minutes at 4°C, the supernatant was centrifuged again at 9,000 x g for 30 minutes a 4°C. The supernatant resulting from this last centrifugation, which corresponds to TS antigen, was frozen in one ml aliquots at -70°C, and then lyophilized (Edward do Brasil, Brasil), and stored at 4°C until use. The antigen extract protein and poly­saccharide contents were determined by BRAD­FORD\textsuperscript{2} method and antrone test\textsuperscript{14}, respectively.

To determine the optimum concentration of antigen to be employed for red cells coating, the block titration was carried out, assaying positive standard CSF obtained from patient with neuro­cysticercosis and negative standard CSF from healthy individual.

CEREBROSPINAL FLUID (CSF) — It were assayed 115 CSF samples from patients with neurocysticercosis confirmed by epidemiologi­cal, clinical and laboratorial data. The control group was consisted of 57 CSF samples from neu­rological patients with diagnosis other than cysticercosis (as meningitis, neurosyphilis, tu­mors, vascular accidents, hydrocephalus, coma), and 32 samples from apparently healthy indivi­duals.

PASSIVE HAEMAGGLUTINATION TEST (PHA) — Formaldehyde-treated group O Rh-hu­man erythrocytes were sensitized with TS anti­gen as previously described by HOSHINO-SHI­MUZI et al.\textsuperscript{6}. Briefly, a 0.5% cell suspension was washed three times in 20 volumes of saline solution, and after last centrifugation the cell pellet was resuspended in an original volume with sali­ne solution. Equal volume of a 1/15,000 dilution of tannic acid diluted in saline solution was ad­ded to the suspension. Tanning was carried out for 10 minutes at 56°C in a water-bath, with fre­quent agitation. After tanning, the cells were wa­shed three times by centrifugation at 800 x g for 10 minutes. Then, they were resuspended to the original volume with solution of TS antigen at the optimal dilution in 0.15M phosphate buffer­red saline solution (PBS) at pH 6.4, and incuba­ted for 50 minutes at 37°C, under gentle shaking. After coating, equal volume of a 0.1% (v/v) guuta­raldehyde solution, diluted in saline solution, was added and the suspension was incubated for 20 minutes again at 37°C, in water-bath. The coated cells were washed three times in PBS and they were made up to one to third of original volume in stabilizing solution\textsuperscript{4}, and lyophilized. For use, the cells were reconstituted to original volume with distilled water: the final concen-
centration of cells was 0.5%. Following the same procedure, the control cells were prepared replacing antigen by pH 6.4 PBS, during antigen coating step.

PHA test was carried out in the polystyrene V-shaped 96-well microtitration plates (Inlab, Brazil) by adding 25 microlitres volumes of a 0.5% cell suspension to a 25 microlitres of neat and a twofold series dilution of CSF in pH 6.4 PBS. Each CSF sample dilution was simultaneously tested with uncoated cells. The plates were shaken for 30 seconds in plate shaker (Titer-tek, Flow Laboratories, USA), and kept in a wet chamber at room temperature. The titration end points were determined after 30 minutes incubation, when the positive and negative controls showed maximum contrast.

STATISTICAL ANALYSIS — The sensitivity, specificity, positive and negative predictive values rates and efficiency of the test were determined according to GALEN & GAMBINO, on the basis of considering 0.1% the prevalence of neurocysticercosis as calculated by SCHENONE ET AL.

RESULTS

Table 1 shows the determination of TS antigen protein and polysaccharide contents.

<table>
<thead>
<tr>
<th></th>
<th>POLYSACCHARIDES</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL SALINE (10 cysticerci/ml)</td>
<td>6.1</td>
</tr>
</tbody>
</table>

According to the results obtained in antigen block titration, the concentration of 60 μg/ml was selected for coating the erythrocytes.

Table II shows the PHA test results assayed in the total of 204 CSF samples including patients with neurocysticercosis and from individuals of control group.

Of the 115 CSF samples from neurocysticercosis patient 94 presented reactivity, providing 81.7% sensitivity with the confidence limit of 95% probability (CL_95%) ranging from 74.5% to 88.9%.

The assay performed in 89 CSF samples from individuals of control group demonstrates non-reactivity in 94.4% at CL_95% ranging from 89.6% to 99.2%.

The positive and negative predictive values and the test efficiency are 1.4%, 99.9% and 94.4%, respectively, when these rates are calculated under the estimation that, the mean rate of prevalence of human neurocysticercosis in Latin America is 100 cases per 100,000 inhabitants, that is to say 1 per 100.

DISCUSSION

The PHA test for cysticercosis has been considered as the more sensitive technique than pre-
TABLE II

Frequency of antibody titres by means of passive haemagglutination test in CSF samples according to three different groups of individuals.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NR</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>64</th>
<th>128</th>
<th>256</th>
<th>512</th>
<th>1024</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>21</td>
<td>8</td>
<td>22</td>
<td>15</td>
<td>22</td>
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<td>13</td>
<td>5</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>115</td>
</tr>
<tr>
<td>b</td>
<td>54</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>57</td>
</tr>
<tr>
<td>c</td>
<td>30</td>
<td>2</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>32</td>
</tr>
</tbody>
</table>

NR = nonreactive

GROUP a = neurocysticercotic patients
GROUP b = patients with neurological clinical symptomatology other than cysticercosis
GROUP c = presumably healthy individuals

The specificity of the standardized haemagglutination test was verified to be high (94.4%), and this is consistent with that was expected for PHA technique. With respect to the five CSF false positive results, two of them derived from patients with presumably viral lymphocytic meningitis, one from patient with hydrocephalus, and two from presumably healthy individuals without any clinical manifestation suggestive of disease.

The low positive predictive value demonstrated in this study resulted from the accounting the low prevalence of disease, according to few available data. In accordance with GALLEN & GAMBINO, it is desirable a more sensitive test than specific one, when the disease is serious, its treatment is uncertain, and its diagnosis has public health value.
The sensitivity of PHA test in detecting specific antibodies in CSF samples, makes it useful for neurocysticercosis diagnosis, mainly in negative cases by means of other less sensitive serological tests routinely used. Also, this test demonstrates to be easy to perform and provides highly reproducible results.

RESUMO

Reação de hemaglutinação passiva para o imunodiagnóstico da neurocisticercose humana. I. Padronização e avaliação do teste de hemaglutinação passiva para a detecção de anticorpos anti-Cysticercus cellulosae.

Foi padronizada e avaliada a reação de hemaglutinação passiva (RHA) para pesquisa de anticorpos específicos, anti-Cysticercus cellulosae, no líquido cefalorraquiano (LCR). Foram utilizadas hemácias humanas O Rh-formolizadas e sensibilizadas com extrato antigenico salino total de císticoerços, ainda pouco estudado. De 115 amostras estudadas de LCR de pacientes com neurocisticercose, 94 foram reagentes, resultando em 81,7% de sensibilidade, com intervalo de confiança de 95% de probabilidade (IC95%) abrangendo de 74,5% e 88,9%. Também foram ensaiadas 89 amostras de LCR de indivíduos do grupo controle, sendo tão reagentes em 94,4%, com IC95 de 89,6% a 99,2%. Os valores preditivos positivo e negativo obtidos para a RHA foram, respectivamente, de 1,4% e 99,9%, considerando a prevalência média de neurocisticercose na América Latina de 0,1%. Os resultados indicam que a RHA como um método simples, altamente reproduzível e moderadamente sensível para a detecção de anticorpos específicos no LCR, porém apropriados para a triagem de infectados.

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