IgG INTRATHECAL SYNTHESIS AND SPECIFIC ANTIBODY INDEX IN PATIENTS WITH NEUROCYSTICERCOSIS

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ABSTRACT - We analyzed cerebrospinal fluid (CSF) and blood serum from 55 patients with neurocysticercosis (NC) at different clinical stages. According to inflammatory activity in the CSF, three stages were identified: (1) reactive, when there was at least an increase in the number of cells; (2) weakly reactive, when significant alterations were found in the CSF, including an increase in gamma globulins, albeit without hypercytosis; (3) non-reactive, when there was neither hypercytosis nor increase in gamma globulins. Nineteen patients had the reactive form; 18 had the weakly reactive form; 18 displayed the non-reactive form. Local immunoproduction was intense in the reactive group, moderate in the weakly reactive group, and absent in the non-reactive group. The specific antibody index was raised in approximately 2/3 of patients with the reactive form, 2/3 in those with the weakly reactive form, and 1/3 in those with the non-reactive form. In conclusion: (1) the classical CSF syndrome in NC can present both in complete and partial modes; (2) local immunoproduction can occur in weakly reactive forms; (3) a raised specific antibody index can occur in the absence of an inflammatory reaction in the CSF.

KEY WORDS: neurocysticercosis, cysticercosis, cerebrospinal fluid, IgG, ELISA, passive hemagglutination, intrathecal synthesis, oligoclonal bands, specific antibody index.

Neurocysticercosis (NC) results from Taenia solium larvae lodged in the central nervous system (CNS). Although it may remain asymptomatic or oligosymptomatic for long periods, NC is usually marked by relapses, in which clinical signs vary in intensity. Diagnosis of NC is based on: clinical signs, neuroimaging (CT and MRI) and cerebrospinal fluid (CSF). CSF changes suggestive for NC were first described by Lange in 1940. They are referred as the CSF syndrome of NC and include: (1) a mild or moderate increase in the number of cells with (2) presence of eosinophils, and (3) presence of anti-Taenia antibodies. New elements were added over following decades, without, however, altering the fundamental characteristics of this CSF syndrome. These changes are induced by parasite antigens and usually are more...
apparent and more severe when affect the CSF space. CSF helps establish the etiological diagnosis (anti-Taenia antibodies immunoproduction) and determine whether the process is active (presence of the syndrome and immunoproduction of non-specific IgGs). Regardless clinical forms and neuroimaging findings, whether active or not, inflammatory changes proper to the CSF syndrome of NC can be detected and are diagnostic parameters.

The objective of this study is to analyze prospectively the behavior of the diagnostic parameters and of the indicators of local inflammatory activity in NC patients, through comparative examination of the CSF and blood serum.

METHOD

We performed a prospective study of 55 patients from the Outpatient Ward for Infectious Diseases of the Nervous System at the São Paulo University Medical School Hospital. This project was approved by the Ethics Committee of the São Paulo University Medical School Hospital. All patients were amply informed as to their participation in the project, and signed the informed consent protocol. All the patients, with well defined clinical complaints, had prev diagnosis of NC confirmed by CSF examination and neuroimaging. We included patients by order of presentation. No restrictions were made as to clinical forms or stage of the disease.

Of the 55 patients, 32 were male (58.2%), 39 were white (70.9%) and 16 brown or black (29.1%). Their ages ranged from 15 to 72 years (average age: 41.9; median age: 42). Distribution by age and color was similar in all three groups. As to gender, men were predominant in the groups presenting the reactive form (68.4%) and the weakly reactive form (61.1%); however, in the non-reactive form, female patients predominated.

All patients were submitted to paired CSF and blood serum examination. The following data were considered: (1) for the CSF exam: pressure; number of cells; cytomorphological profile (with emphasis to the presence of neutrophils and eosinophils); glucose content; adenosine-deaminase (ADA) enzyme activity; (2) for the simultaneous CSF and blood serum test: total proteins; protein electrophoresis (isoelectrofocusing); albumin and IgG concentrations (nephelometry); presence of specific antibodies by: complement fixation test (CF), indirect immunofluorescence test (IFT), passive hemagglutination test (PHA), enzyme-linked immuno-sorbet assay (ELISA) and immunoblotting.

The Kölmer technique, modified by Spina-França, was used for CF. IFT was processed following the technique of Camargo et al., modified by Livramento. The PHA reaction was carried out in compliance with the technique of Ferreira et al. The ELISA reaction was processed in accordance with the technique of Costa et al. Immunoblotting was performed as per Vaz et al. IFT and immunoblotting were used only as qualitative reactions.

The CSF/serum albumin quotient was used to evaluate the integrity of the CSF-blood barrier. To characterize local immunoproduction we used Link & Tibbling IgG indices, Tourtellote’s daily IgG synthesis, Thompson’s nomogram (IgG quotient versus albumin quotient) and protein isoelectrofocusing. Anti-Taenia specific antibody indices were calculated by the method established by Reiber and Felgenhauer, using an ELISA assay. This method was used to evaluate the ratio between CSF/serum quotient for specific antibodies and total IgG.

Three forms were considered, in accordance with the inflammatory activity recorded in the CSF examination: (1) a reactive form, when there was an increase in the number of cells, regardless of intensity, and regardless the occurrence of other CSF changes; (2) a weakly reactive form, when there were significant alterations in the CSF; including an increased gamma globulin content, albeit without hypercytosis; and (3) a non-reactive form, when there was neither hypercytosis nor an increased gamma globulin content, although mild isolated biochemical alterations could be found. Nineteen patients had the reactive form; 18 had the weakly reactive form; 18 had the non-reactive form.

Statistical analysis used the following tests: Chi-square (or Fisher’s exact test), the Mann-Whitney U test, and the Kruskall-Wallis test. SPSS 7.5 for Windows was used for statistical study (SPSS Inc, Chicago, Illinois, USA). For the entire test, alpha = 0.05 was established.

RESULTS

Table 1 shows the general data for the CSF. Hypertension is observed with similar frequency in all three groups studied. The other variables present significant fluctuation for the three groups. ADA activity levels are significantly raised in the reactive group patients.

Table 2 displays the rates of IgG alterations and IgG immunoproduction rates, as well as their respective p values. Due to the classification criterion adopted, the frequency of alterations for all the variables analyzed was significantly different in the three groups (p < 0.0001). For this reason statistical comparison was only carried out for the reactive and weakly reactive forms.

Table 3 shows estimators and descriptive data for IgG and quantitative immunoproduction indices as well as the statistical comparison between the three groups considered. As with the results in Table 2, all variables presented significantly different results (p < 0.0001), owing to the classification criterion that was used. Therefore we consider p values relating to the comparison between the 2 groups where there is an inflammatory reaction of the CSF.

Table 4 shows frequencies for specific reactant
immunological tests for cysticercosis, in the CSF and the blood serum. Table 5 shows the medians for the different immunological tests, as well as the p and respective level of significance for CSF and serum.

Table 6 displays the specific antibody indices of Reiber and Felgenhauer, with regard to the frequency with which they were elevated and to the values of their medians. If we consider the frequency only in the reactive and weakly reactive groups, there was no statistically significant difference (p=0.40). Considering the results case by case in the same groups, the values for specific antibody indices also showed no significant variation (p=0.41).

**DISCUSSION**

The CSF syndrome of NC is expressed differently in the three groups: completely in the reactive group; partially in the weakly reactive group, and virtually absent in the non-reactive group (Tables 1 to 5). NC does not always present inflammatory changes in the CSF26, 27, as can be seen in Table 1: (1) intracranial hypertension is observed with similar frequency in all three groups, suggesting the occurrence of the mass effect even in patients with the form that is non-reactive to the CSF exam; (2) an increase in proteins and a decrease in the glucose levels are observed, albeit less markedly, in significant percentages in the non-reactive group.

The inflammatory alterations in NC patients (Ta-
tables 2 and 3) present different behavior when one considers the frequency of alterations or of estimators relating to local IgG immunoproduction. Oligoclonal bands occurred with significantly different frequency in the reactive and weakly reactive groups; the increase in IgG, the alterations in Thompson's nomogram and the increase in the quantitative immunoproduction indices occurred with similar frequency in the reactive and weakly reactive groups. The reactive and weakly reactive groups presented a statistically significant difference in IgG intrathecal synthesis estimators for all the variables analyzed. These findings allow us to affirm that, for the quantitative variables, the inflammatory reaction in the CSF of NC patients occurs more intensely in the reactive forms, although the rate is similar for both groups. However, oligoclonal bands occur more frequently in the reactive form.

Analyzing the results of the specific immunological reactions (Tables 4 and 5), the following facts are striking: (1) in the non-reactive form, the ELISA test is relatively less sensitive; (2) in the non-reactive form, PHA is reactant with a greater frequency than the other reactions. This suggests that PHA may present greater sensitivity than other reactions in the forms of NC that are not accompanied by inflammatory changes in the CSF; (3) immunological reactions for cysticercosis present a higher degree of sensitivity when used together; the association of at least two reactions is important (PHA and ELISA); (4) when performed only on serum, immunological reactions have increased sensitivity, but run the risk of presenting decreased specificity.

The index of Reiber and Felgenhauer specific antibodies enables the diagnosis of CNS involvement in a high percentage of patients. As is classically known, a high index is unrelated to the activity of the disease. Disease activity is, however, best characterized by the presence of a generalized inflammatory reaction and the presence of local immunorelease of IgG (quantitative and/or qualitative criteria). Owing to the fact that NC is a chronic disease with recurrent acute episodes, the antibodies studied are from the IgG class, although there may be alterations of antibodies from classes IgM, IgA and IgE. Actually, in the non-reactive form (in which there is no local immunoproduction), 1/3 of patients presented an increase in the index of specific antibodies. The numerical values of these indices are similar to those found in the reactive and weakly reactive forms of NC (Table 6).

Sotelo and subsequently Del Brutto's descriptions of the active and inactive forms of NC enable the formation of NC groups that may benefit and groups that may not benefit from specific treatment. The detection in the CSF of antigens released by the parasite contributes for this purpose and is an additional marker to the study and clarification of the parasite-host relationship, as our research group recently showed. Also, the recognition of different modes of presentation of the inflammatory reaction of the CSF in NC patients aims, presented in this study, provides new information so as to supplement current criteria in order to distinguish between the active and inactive forms of NC.

In conclusion: (1) it is possible to characterize variable expressivity in the classical CSF syndrome in NC patients, emphasizing either the diagnosis and/or the activity of the disease. This enables delimitation of differentiated at-risk groups among patients infected with NC; (2) in NC, local immunoproduction phenomena occur significantly even in forms whose CSF is weakly reactive; (3) an increase in specific antibody indices can be detected, and hence it is possible to establish the immunological diagnosis of the disease even when inflammatory alterations are not found in the CSF.

Table 5. Specific IgG antibodies to cysticercosis (median).

<table>
<thead>
<tr>
<th></th>
<th>Reactive form (n=19)</th>
<th>Weakly reactive form (n=18)</th>
<th>Non-reactive form (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PHA</td>
<td>1/128</td>
<td>1/24</td>
<td>1/4</td>
</tr>
<tr>
<td>ELISA</td>
<td>111.0</td>
<td>54.0</td>
<td>0</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.001 S</td>
<td>&lt;0.001 S</td>
<td>&lt;0.001 S</td>
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</tbody>
</table>

Table 6. Reiber & Felgenhauer antibody index in patients with neurocysticercosis.

<table>
<thead>
<tr>
<th></th>
<th>Reactive form (n=19)</th>
<th>Weakly reactive form (n=18)</th>
<th>Non-reactive form (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased (%)</td>
<td>78.9</td>
<td>66.7</td>
<td>33.3</td>
</tr>
<tr>
<td>Median</td>
<td>2.9</td>
<td>8.4</td>
<td>5.0</td>
</tr>
<tr>
<td>p</td>
<td>0.009 S</td>
<td>0.62 NS</td>
<td>0.001 S</td>
</tr>
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REFERENCES


