Serodiagnosis of Human Cysticercosis by Using Antigens from Vesicular Fluid of *Taenia crassiceps* Cysticerci

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Received 2 April 2001/Returned for modification 27 July 2001/Accepted 20 August 2001

Neurocysticercosis (NC), caused by the presence of *Taenia solium* metacestodes in tissues, is a severe parasitic infection of the central nervous system with universal distribution. To determine the efficiency of enzyme-linked immunosorbent assay (ELISA) and immunoblot with antigens of *T. crassiceps* vesicular fluid (Tcra) compared to standard techniques (indirect immunofluorescence test [IFT] and complement fixation test [CFT]) using *T. solium* cysticerci (Tso) for the serodiagnosis of NC, we studied serum samples from 24 patients with NC, 30 supposedly healthy individuals, 76 blood bank donors, 45 individuals with other non-NC parasitoses, and 97 samples from individuals screened for cysticercosis serology (SC). The sensitivity observed was 100% for ELISA-Tso and ELISA-Tcra, 91.7% for the IFT, and 87.5% for the CFT. The specificity was 90% for ELISA-Tso, 96.7% for ELISA-Tcra, 50% for IFT, and 63.3% for CFT. The efficiency was highest for ELISA-Tcra, followed by ELISA-Tso, IFT, and CFT. Of the 23 samples from SC group, which were reactive to ELISA-Tso and/or ELISA-Tcra, only 3 were positive to immunoblot-Tcra (specific peptides of 14- and 18-kDa) and to glycoprotein peptides purified from Tcra antigen (gp-Tcra), showing the low predictive value of ELISA for screening. None of the samples from the remaining groups showed specific reactivity in immunoblot-Tcra. These results demonstrate that ELISA-Tcra can be used as a screening method for the serodiagnosis of NC and support the need for specific tests for confirmation of the results. The immunoblot can be used as a confirmatory test both with Tcra and gp-Tcra, with the latter having an advantage in terms of visualization of the results.

MATERIALS AND METHODS

**Samples.** The serum samples used in this study were obtained from 24 patients with NC (NC), 30 supposedly healthy individuals (C), 76 blood bank donors (BB), and 45 individuals with other non-NC parasitoses (OP). In addition, 97 serum samples from individuals medically screened for cysticercosis serology (screening serologic [SC]) were used. The present study was approved by the Ethics Committee for the analysis of Research Projects of the Clinical Director’s Office of the Hospital 072/97, according to Resolution 196/96 of the National Health Council, Ministry of Health, Brazil.

**Antigens.** Antigen extracts were obtained from the vesicular fluid of *T. crassiceps* cysticerci (Tcra) and from the membrane and scolex of *T. solium* cysticerci (Tso) as described before (2). The Tcra antigen was purified in order to obtain glycoprotein peptides (gp-Tcra) of low molecular mass (18 and 14 kDa) by elution in preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (PrepCell 491; Bio-Rad Laboratories, Inc.). The fractions were collected and analyzed by silver stain. The fractions of interest were pooled and elution in preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (PrepCell 491; Bio-Rad Laboratories, Inc.). The fractions were collected and analyzed by silver stain. The fractions of interest were pooled and concentrated.

**IFT and CFT.** Antibody detection by the indirect immunofluorescence test (IFT) and complement fixation test (CFT) was adapted for use in serum samples according to the protocols of the Neurology Investigation Center of the Faculty of Medicine of São Paulo University (22).

ELISA-Tcra, ELISA-Tso, and immunoblot-Tcra. *T. solium* anti-cysticercus immunoglobulin G (IgG) antibodies in serum samples diluted at 1:100 were detected by ELISA (ELISA-Tcra, ELISA-Tso, and immunoblot-Tcra). The Tcra antigen was purified in order to obtain glycoprotein peptides (gp-Tcra) of low molecular mass (18 and 14 kDa) by elution in preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (PrepCell 491; Bio-Rad Laboratories, Inc.). The fractions were collected and analyzed by silver stain. The fractions of interest were pooled and concentrated.

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determined by ELISA using Tcra and Tso antigens and by immunoblot using Tcra antigens, as described previously (2). The reactivity index (RI) was calculated by using the obtained absorbance divided by the cutoff (mean absorbance for the control group plus two standard deviations).

**Immunoblot gp-Tcra.** Some ELISA and/or immunoblot-Tcra-reactive samples were processed for the identification of specific peptides in the gp-Tcra antigen (immunoblot gp-Tcra). Positive, negative, and background controls were included in each test. gp-Tcra antigen at 1:50 dilution was fractionated by SDS-PAGE on a 15% gel (13) and transferred electrophoretically to 0.22-μm (pore-size) nitrocellulose membranes (Immobilon-PSQ Transfer Membrane; Millipore) (23) that were cut into 3- to 4-mm-wide strips. Phosphate-buffered saline at 0.01 M (pH 7.2; 0.0075 M Na₂HPO₄, 0.025 M NaH₂PO₄, and 0.14 M NaCl) containing 0.05% Tween 20 was used for the washes and for the preparation of skim milk. The strips were blocked for 2 h with 5% milk, and the serum samples were diluted 1:100 with 1% milk and incubated for 18 h at 4°C. The conjugate used was alkaline phosphatase-goat anti-human IgG (Sigma Chemical Co.) incubated for 2 h. Reactive fractions were developed with nitroblue tetrazolium-5-bromo-4-chloro-3-indolylphosphate (NBT-BCIP; Sigma Chemical Co.). Molecular weight markers were used for calculating the molecular mass of the reactive fractions (14).

**RESULTS**

The results obtained for all sample groups by all tests are presented in Table 1.

Considering a cutoff of 1:20, IFT showed reactivity in 22 (91.7%) samples from patients with NC, 15 (50%) samples from supposedly healthy individuals, 17 (22.4%) samples from blood bank donors, 23 (51.1%) samples from individuals with other parasitoses, and 57 (58.8%) samples from screening serologic group. The reactivity detected with CFT, considering a reactivity threshold of 1:32, was 87.5% (21 of 24) for patients with NC, 36.7% (11 of 30) for supposedly healthy individuals, 21.0% (16 of 76) for blood bank donors, 35.5% (16 of 45) for individuals with other parasitoses, and 22.7% (22 of 97) for the screening serologic group.

The results obtained by ELISA-Tso and -Tcra with serum samples from patients with NC, from subjects medically screened for SC, supposedly healthy individuals (C), blood bank donors (BB), and individuals with other non-NC parasitoses (OP) are shown in Fig. 1.

ELISA-Tso showed RIs of 1.4 to 6.7 (3.9 ± 1.7) for the samples from patients with NC (24 of 24, RI ≥ 1.0), 0.2 to 1.7 (0.7 ± 0.3) for supposedly healthy individuals (6 of 30, RI ≥ 1.0; 3 of 30, RI ≥ 1.2), 0.3 to 2.6 (0.8 ± 0.4) for blood bank donors (22 of 76, RI ≥ 1.0; 12 of 76, RI ≥ 1.2), 0.3 to 3.6 (0.8 ± 0.5) for individuals with other parasitoses (12 of 45, RI ≥ 1.0, 8 of 45, RI ≥ 1.2), and 0.2 to 6.5 (0.8 ± 0.9) for the screening serologic group (20 of 97, RI ≥ 1.0; 16 of 97, RI ≥ 1.2).

The RIs detected with ELISA-Tcra were 1.2 to 9.7 (5.3 ± 2.6) for patients with NC (24 of 24, RI ≥ 1.0), 0.3 to 3.3 (0.7 ± 0.5) for supposedly healthy individuals (5 of 30, RI ≥ 1.0; 1 of 30, RI ≥ 1.2), 0.3 to 7.0 (1.0 ± 1.1) for blood bank donors (20 of 76, RI ≥ 1.0; 14 of 76, RI ≥ 1.2), 0.3 to 6.9 (1.2 ± 1.5) for individuals with other parasitoses (15 of 45, RI ≥ 1.0; 11 of 45, RI ≥ 1.2), and 0.2 to 8.0 (0.9 ± 1.2) for screening serologic group (23 of 97, RI ≥ 1.0; 13 of 97, RI ≥ 1.2).

**Table 1. ELISA, IFT, and CFT results**

<table>
<thead>
<tr>
<th>Group</th>
<th>ELISA-Tcra</th>
<th>ELISA-Tso</th>
<th>IFT</th>
<th>CFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>0.348–2.941 (1.586 ± 0.790)</td>
<td>0.549–2.678 (1.576 ± 0.664)</td>
<td>0–320 (65 ± 28)</td>
<td>0–256 (42 ± 3)</td>
</tr>
<tr>
<td>SC</td>
<td>0.074–2.387 (0.276 ± 0.351)</td>
<td>0.062–2.595 (0.335 ± 0.375)</td>
<td>0–320 (25 ± 25)</td>
<td>0–128 (6 ± 4)</td>
</tr>
<tr>
<td>C</td>
<td>0.093–0.661 (0.272 ± 0.138)</td>
<td>0.091–0.994 (0.214 ± 0.169)</td>
<td>0–80 (17 ± 19)</td>
<td>0–64 (12 ± 3)</td>
</tr>
<tr>
<td>BB</td>
<td>0.079–2.111 (0.311 ± 0.335)</td>
<td>0.121–1.025 (0.330 ± 0.168)</td>
<td>0–40 (12 ± 15)</td>
<td>0–64 (9 ± 3)</td>
</tr>
<tr>
<td>OP</td>
<td>0.079–2.066 (0.376 ± 0.453)</td>
<td>0.114–1.456 (0.328 ± 0.225)</td>
<td>0–320 (20 ± 23)</td>
<td>0–256 (13 ± 4)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In NC, most of the antibodies found in CSF are intrathecally synthesized, with a smaller proportion coming from peripheral blood due to blood-brain barrier rupture (4). Several authors have detected IgG antibodies in CSF and/or serum from patients with NC, especially when the parasite is in the phase of...
degeneration and there is an increased immune inflammatory host response (3, 4, 5).

The ELISA and immunoblot test has been used in the study of NC, and different indices of sensitivity and specificity have been observed depending on the antigen preparation, the type and severity of the lesions, and the inflammatory reaction surrounding the parasite (2, 3, 7, 10, 11, 12, 16, 17, 24, 26, 27).

The results obtained here with ELISA-Tcra showed slightly higher readings than those obtained with ELISA-Tso regardless of the group studied (Fig. 1), as previously described (2). All of the tests performed always showed higher concordancy than copositivity indices for the samples from individuals with other parasitoses and from blood donors and for the screening serologic group. Better agreement indices were observed when a cutoff of 1.2 was considered for the ELISA.

The choice of the cutoff for ELISA can determine a better efficiency of the test. An RI of ≥1.2 resulted in a higher specificity without altering the sensitivity. Considering the immunoblot as confirmatory for the specificity of the antibodies, the use of an RI of ≥1.2 for ELISA-Tcra detected the three samples in the SC group. Thus, for clinical laboratory use, a high cutoff is preferable in order to reduce the number of confirmatory tests. On the other hand, studies using the test for epidemiologic survey purposes are still necessary.

The sensitivity and specificity indices showed a better efficiency of ELISA-Tcra, followed by ELISA-Tso, IFT, and CFT.

![Graph showing RI obtained in ELISA with Tso and Tcra in serum samples from 24 patients with NC, 97 subjects submitted to medical screening for SC, 30 supposedly healthy individuals (C), 76 blood bank donors (BB), and 45 individuals with other non-NC parasitoses (OP).](http://cvl.asm.org/)

FIG. 1. RI obtained in ELISA with Tso and Tcra in serum samples from 24 patients with NC, 97 subjects submitted to medical screening for SC, 30 supposedly healthy individuals (C), 76 blood bank donors (BB), and 45 individuals with other non-NC parasitoses (OP).
TABLE 2. Assay sensitivities and specificities

<table>
<thead>
<tr>
<th>Test</th>
<th>NC group (n = 24)</th>
<th>C group (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Sensitivity</td>
<td>RI</td>
</tr>
<tr>
<td>ELISA-Tso</td>
<td>100</td>
<td>≥1.0</td>
</tr>
<tr>
<td>ELISA-Tcra</td>
<td>100</td>
<td>≥1.0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>≥1.2</td>
</tr>
<tr>
<td>IFT</td>
<td>91.7</td>
<td></td>
</tr>
<tr>
<td>CFT</td>
<td>87.5</td>
<td></td>
</tr>
</tbody>
</table>

* Sensitivity and specificity of ELISA with Tso and Tcra, of IFT, and of CFT with samples from patients with NC and from supposedly healthy individuals (C), with RI cutoffs of 1.0 and 1.2 for the ELISA.

The sensitivity (100%) of ELISA applied to serum samples showed that the test can be used as a screening method for the serodiagnosis of NC as long as positive cases are confirmed by immunoblot. Of the 23 samples in the SC group that were reactive to ELISA-Tso and/or -Tcra (RI ≥ 1.2), only 3 were positive by immunoblot-Tcra and immunoblot gp-Tcra, showing the low predictive value of ELISA for screening. The very low frequency of positivity in the SC group (3 of 97 [3%]) was the low predictive value of ELISA for screening. The very low positive by immunoblot-Tcra and immunoblot gp-Tcra, showing reactive to ELISA-Tso and/or -Tcra (RI ≥ 1.2), only 3 were positive by immunoblot-Tcra and immunoblot gp-Tcra, showing the low predictive value of ELISA for screening. The very low frequency of positivity in the SC group (3 of 97 [3%]) was expected if we assume that the medical request for a test is frequently used to exclude the hypothesis of NC, since the most probable cases are referred to a neurology clinic which will use neuroimaging and CSF exams. These results emphasize the need for specific tests for confirmation of the results, as also reported by others (24). In a previous study we demonstrated the viability of the use of ELISA-Tcra in serum samples, as well as the need to confirm positive cases by immunoblot (2). Other authors have used only the immunoblot for NC diagnosis (24), but it is expensive for use in developing countries.

Although the results of immunoblot demonstrated that both the Tcra antigen and the purified gp-Tcra antigen can be used to confirm the positivity of ELISA, the use of the gp-Tcra antigen has advantages in terms of interpretation of the results due to its constitution (only two peptides of 14 and 18 kDa, both of them specific).

The preparation of purified antigens (Fig. 2) could be useful for ELISA, with a probable persistence of sensitivity and increased specificity. So far we have not obtained good results with gp-Tcra to sensitize the polystyrene plates (data not shown), probably because of the impairment of adsorption of glycoprotein structures, which is overcome when nitrocellulose is used in the immunoblot. A further perspective is the obtaining of recombinant antigens and/or synthetic peptides from *T. crassiceps*. This approach would provide a more consistent and reproducible supply of protein antigens for use in NC immunodiagnosis, as recently described with *T. solium* cysticerci (8, 9).

ACKNOWLEDGMENTS

Part of this work was supported by FAPESP (97-02245-6) and PIQDT/CAPES fellowship (Ednêa Casagrande Bueno). We are indebted to Regina H. S. Peralta for technical assistance with the preparation of some tests for confirming the positivity of ELISA, the use of the gp-Tcra antigen has advantages in terms of interpretation of the results due to its constitution (only two peptides of 14 and 18 kDa, both of them specific).

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REFERENCES