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SERODIAGNOSIS OF NEUROCYSTICERCOSIS IN PATIENTS WITH EPILEPTIC SEIZURE USING ELISA AND IMMUNOBLOT ASSAY

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SUMMARY

Sera from 88 patients from Santa Catarina and São Paulo states of Brazil, with epileptic seizures who underwent cerebral computed tomography (CT) were analyzed for the detection of antibodies to *T. solium* cysticercus by ELISA and Immunoblot (IB) with the following antigens: *Taenia solium* cysticercus total saline (Tso), *Taenia crassiceps* cysticercus vesicular fluid (Tcra-vf) and *T. crassiceps* cysticercus glycoproteins (Tcra-gp). ELISA carried out with Tso, Tcra-vf and Tcra-gp antigens showed 95%, 90% and 80% sensitivities, respectively, and 68%, 85% and 93% specificities, respectively. In the epileptic patients group, ELISA positivity was 30%, 51% and 35% with Tso, Tcra-vf and Tcra-gp antigens respectively. Considering the IB as the confirmatory test, the positivity was 16% (14/88) in the epileptic patients total group and 22% (12/54) in the epileptic patients with positive CT and signals of cysticercosis. We found a significant statistical correlation among ELISA or IB results and the phase of the disease when any antigens were used ($p < 0.05$). We emphasize the need to introduce in the laboratory routine the search for neurocysticercosis (NC) in patients presenting with epileptic seizures because of the high risk of acquiring NC in our region and its potential cause of epilepsy.

KEYWORDS: *Taenia solium*; *Taenia crassiceps*; Cysticercosis; Serological diagnosis; ELISA; Immunoblot.

INTRODUCTION

Neurocysticercosis (NC), an infection caused by *Taenia solium* cysticerci lodged in the central nervous system (CNS), is recognized as a leading cause of symptomatic epilepsy in developing countries[3,19]. The etiologies of epilepsy are many but Brazilian studies using computed tomography (CT) found that almost 30% of all epilepsy is related to cysticercosis[2]. However most of patients with NC are asymptomatic[6]. When symptomatic, NC can also be presented as intracranial hypertension syndrome, cysticercal meningitis, hydrocephalus and signals of spinal cord compression. This clinical pleomorphism is mainly related to the number, size, type, developmental stage and site of cysts in the nervous system, as well as the host inflammatory response[12]. Epileptic seizures seem to be more common in patients presenting with multiple lesions[14] and these may occur at the time of cyst degeneration[7].

The diagnosis of NC relies on clinical, epidemiological and laboratory criteria and on imaging examinations such as CT and magnetic resonance imaging (MRI)[11]. The main limitation of immunological techniques is the difficulty in obtaining antigens from *T. solium* larvae in a large amount, enough to undergo purification processes, which are need to reduce the nonspecific or cross-reactive fractions from the total antigenic extracts. Some studies demonstrated that *T. solium* from pig infection and *T. crassiceps* which can be maintained in mice, share antigenic components, including those of low molecular mass peptides (18 and 14 kDa)[8,15]. *T. crassiceps* larvae, which are easily maintained in the laboratory by intraperitoneal passage through female BALB/c mice, were shown to be an important alternative source of such antigen components[5,17,22].

In order to evaluate the performance of different antigens from *T. solium* and *T. crassiceps* cysticerci in the diagnosis of neurocysticercosis, sera from epileptic patients were analyzed for detection of antibodies IgG to *T. solium* cysticerci by ELISA and immunoblot assay. Also, the potential capability of these techniques using those antigens in order to discriminate the phases of the NC in epileptic patients was investigated.

MATERIALS AND METHODS

Serum samples. Epileptic patients group: A total of 88 serum samples were collected from patients suffering from epilepsy who underwent CT. Fifty four (54) out of 88 had suggestive signals of neurocysticercosis (12 with active or mixed lesions and 42 with calcified lesions), whereas 34 remainder patients showed negative results in the CT exam. Eighty patients were from the *Clinica Multidisciplinar de Epilepsia, Estado de Santa Catarina, SC, Brasil* and eight patients

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were from the *Hospital das Clínicas (HC), Universidade de São Paulo, SP, Brasil (HC-USP)*.

Control Groups: Twenty serum samples from patients with NC confirmed by imaging exams (CT and/or MRI) and serologic assays (ELISA and IB), obtained from BUENO's study[5], were used as positive control group (*NC group*). Fifty three serum samples from blood donors were used as negative control group (*non NC group*), distributed as follow: 20 samples were kindly supplied by *Biolab-Mérieux Laboratory SA, São Paulo, Brazil* and 33 samples were colletecd from Hemotherapy Service of *Hospital Universitário, Universidade Federal de Santa Catarina, Brazil*.

Inform consent was obtained from all adult participants and from parents of minors and the research was approved by the Ethics Committee of *Universidade Federal de Santa Catarina, SC, Brazil* (process number 007/98) and *Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, SP, Brazil* (process number 035/00) according to Resolution 196/96 of the National Health Council, Ministry of Health, *Brasília, Brazil*.

Parasite antigens: A total saline extract of *T. solium* cysticerci (Tso) and vesicular fluid of *T. crassiceps* cysticerci (Tcra-vf) were obtained as described before[16]. Purified glycoproteins of *T. crassiceps* cysticercus (Tcra-gp) were isolated by affinity chromatography, using a Concanavalin A-Sepharose 4B column[17].

Enzyme-linked immunosorbent assay (ELISA): The ELISA was performed according to previously standardized protocols: with Tso and Tcra-vf[5] and with Tcra-gp[15]. Determination of the cut-off values was based on the analysis of the diagnostic efficiency, according to the Youden index[23] calculated for the absorbance values of the control groups (mean ± *n* standard deviations). Reactivity index (RI) values were calculated by dividing the mean absorbance value of two readings from each sample assayed by the cut-off value corresponding to each antigen used. Samples with RI > 1 were classified as reactive. Among 20 samples from *NC group*, 19 (95%), 18 (90%) and 16 (80%) gave positive results in ELISA using respectively, Tso, Tcra-vf and Tcra-gp. Among 53 serum samples from *Non NC Group*, only 20 samples from *Biolab-Mérieux Laboratory SA, São Paulo, Brazil* were used to calculate the cut-off value and three of them gave positive results in ELISA using Tcra-vf.

Immunoblot assay (IB): The IB was performed with the Tcra-vf antigen, as described before[5]. In order to define the positivity criterion for diagnosis of cysticercosis by the IB, we studied the reactivity to the Tcra-vf peptide antigens of 20 samples from *NC group* and 20 samples from *Biolab-Mérieux Laboratory SA, São Paulo, Brazil*. The molecular masses of the antigenic fractions were calculated on the basis of molecular masses standards and the respective relative fronts (Rf), with the aid of the Excel program. All 20 samples from *NC group* reacted to the antigenic peptides 18-14 kDa of Tcra-vf and conversely, none of the 20 samples from *Non NC Group* assayed reacted to the same antigenic peptides. Thus we assumed that any reaction against the low molecular mass range of 18-14 kDa of Tcra-vf antigen should be considered specific.

Statistical analysis. To determine the proportion or association the Q-square test was used or the Fisher exact test. The significance level adopted in the whole study was 5%[10].

RESULTS

The ELISA and IB assay results obtained for serum samples from epileptic patients according to the CT exam result, type of lesion and antigen used are presented in Table 1.

The ELISA results with sera from the control groups and from the epileptic patients, according to the lesion type detected in the CT exam and according to the antigen used, are presented in Figure 1. Among 53 samples from *Non NC group*, 16 (30%), 8 (15%) and 5 (9%) presented positive results with ELISA using Tso, Tcra-vf and Tcra-gp, respectively (Fig. 1), and three (6%) were positive in the IB assay (data not shown). The IB assay with serum samples from epileptic patients is shown in Figure 2. Nonspecific reactions were observed with molecular sizes higher than the specific (18-14 kDa) peptides.

The results of the serologic techniques (ELISA and IB) showed a significant statistical correlation with the type of lesion (active/mixed or calcified) or absence of lesions detected by the CT exams ($p < 0.05$).

Table 1
Positive results of enzyme-linked immunosorbent assay (ELISA) and immunoblot assay (IB) in epileptic patients from Santa Catarina and São Paulo states, Brazil, according to Computed Tomography (CT) exam, type of lesion and antigen used

		ELISA			IB
	No. tested	Tso n+ (%)	Tcra-vf n+ (%)	Tcra-gp n+ (%)	Tcra-vf n+ (%)
Active/mixed lesions	12	10 (83)	12 (100)	12 (100)	11 (92)
Calcified lesions	42	8 (19)	18 (43)	10 (24)	1 (2)
Total positive CT	54	18 (33)	30 (56)	22 (41)	12 (22)
Negative CT	34	8 (24)	15 (44)	9 (26)	0 (0)
Total epileptics	88	26 (30)	45 (51)	31 (35)	14 (16)

Tso = *Taenia solium* cysticercus total saline; **Tcra-vf** = *Taenia crassiceps* cysticercus vesicular fluid; **Tcra-gp** = *T. crassiceps* cysticercus glycoproteins purified by affinity chromatography with lectin.

DISCUSSION

The diagnosis of NC is a relevant subject because of its immunopathological complexity, being one of the most frequently studied parasitosis in recent years. Epilepsy is the more common manifestation of NC but as it may have other cause than NC, the serological techniques emerge as of great importance to help the diagnosis of this parasitosis, together with the image exams. The symptoms of NC are nonspecific and depends on the number of cysticercus alive, in degeneration or already calcified, the location of the lesions and the host's immune response. The results of serologic techniques are also directly related to these data and could explain why their efficiency varies according to authors.

In our study, ELISA carried out with Tcra-vf or Tcra-gp showed a good screening and diagnostic performance for the diagnosis of cysticercosis in

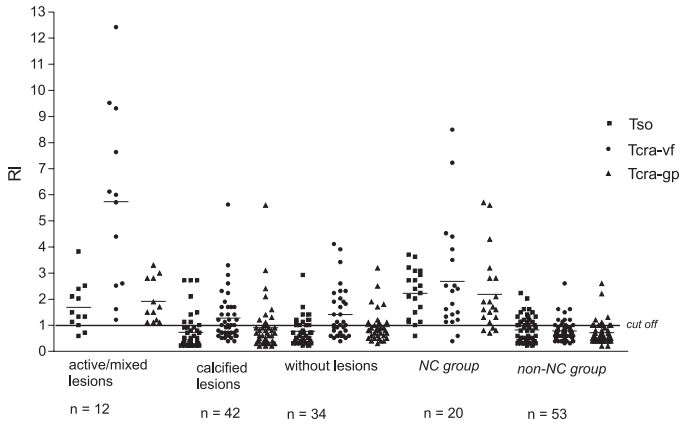

Fig. 1 - Reactivity Index values (RI) of ELISA using: *Taenia solium* cysticercus total saline (Tso); *Taenia crassiceps* cysticercus vesicular fluid (Tcra-vf) and *T. crassiceps* cysticercus glycoproteins purified by affinity chromatography with lectin (Tcra-gp) with the serum samples from epileptic seizure patients (n = 88), according to the lesion type detected in the computed tomograph exam and from the *control groups* (*NC group = Neurocysticercosis group; non-NC group = Normal group*). The bars mean average RI values.

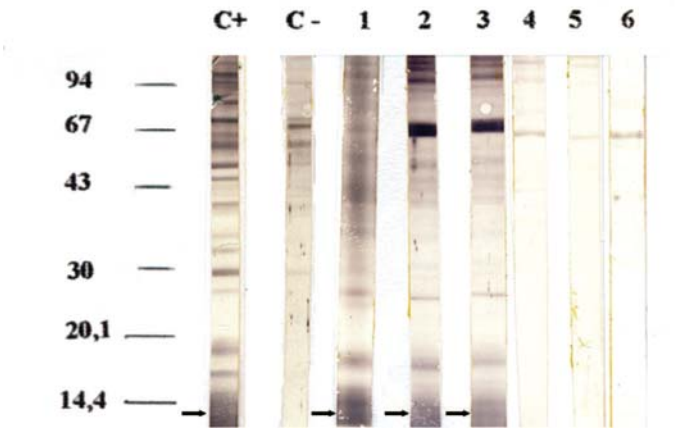

Fig. 2 - Immunoblot assay using vesicular fluid of *Taenia crassiceps* cysticerci and serum samples from epileptic patients. C+ *positive control*; C- *negative control*; positive results (lanes 1 to 3) and negative results (lanes 4 to 6). Molecular masses (kDa) are shown at the left. The arrows show specific reactions with 18-14 kDa peptides.

patients with proved NC (active/mixed lesions). The IB also showed a very sensitive method in the diagnosis of NC from viable cysts, though it failed to detect antibodies in a single serum sample from a patient with mixed lesion.

The results obtained by serologic techniques in the study of sera from patients with calcified lesions are difficult to be interpreted once there are different etiologic agents which can cause epilepsy. As only one sample from calcified lesions was reactive to the IB (with high RI value), we suppose that in our sampling either the antibody levels were lower than the sensitivity of the IB or the ELISA results were false positive. In the *non NC group* we obtained positive results mainly with the Tso antigen. The specificity of the ELISA with Tcra-vf has been reported as 80% and 96% by BUENO *et al.*[5] and BRAGAZZA *et al.*[4], respectively, while PERALTA *et al.*[17] reported 98% specificity for ELISA with 14 kDa glycoprotein of *T. crassiceps*. In this study the Tcra-gp antigen showed similar sensitivity and better specificity than that of Tcra-vf, so Tcra-gp seems to be the best choice to be attempt in order to improve the specificity of ELISA for screening purpose and efforts are underway through purification of the specific and immunodominant antigens. More recently, ELISA using 18-14 kDa proteins from *T. crassiceps* cysticercus obtained by immunoaffinity chromatography showed a good performance and high specificity for serum samples becoming not necessary the use of IB as confirmatory test[9].

According to BERN *et al.*[1], the IB developed by the *Centers for Disease Control - CDC*, Atlanta, USA using purified glycoprotein[21] is highly sensitive in patients with active multiple lesions and conversely, is less sensitive in patients with single lesion and with those with calcified lesions. The majority of the samples analyzed in this study belongs to patients with calcified lesions and information about number of cysts lodged in the CNS of those with enhancing intracranial lesions was not available.

The positivity of the ELISA in the sera from epileptic patients without detectable lesions by CT exam may be attributed to the low sensitivity of the CT to detect microlesions; presence of cysticerci causing no successful infection, but able to produce antibodies and the lack of specificity of the serologic technique. We have previously studied sera from patients with different helminthiases against different helminth antigens used in ELISA and IB: cross-reactivities occurred mostly with the use of antigens belonging to phylogenetically related parasite species[13].

The results of ELISA and IB had a good correlation with CT findings (active, calcified or absence of lesions), that is to say these techniques presented good diagnostic features to discriminate the phase of the disease ($p < 0.05$). As detection of calcified cysts showed to be relatively low, a positive result is highly indicative of active disease, an important fact to make a decision about a treatment.

The challenges for the population control of cysticercosis and epilepsy in the developing countries depend on a reliable and low cost immunological techniques which should be performed with serum samples.

Because of the socioeconomic peculiarities with disseminated pig rearing, the south of Brazil is considered area of high risk of acquiring cysticercosis. TREVISOL-BITTENCOURT *et al.*[20] reported 24% of NC cases in epileptic patients who underwent CT during 1995 and 1996, admitted to the Chapecó Hospital, west region of Santa Catarina (SC). In a retrospective study conducted in an Epilepsy Clinic from Florianópolis, SC, RIGATTI & TREVISOL-BITTENCOURT[18] reported NC as one of the most frequent and relevant etiology of epilepsy.

This study represents the first serology survey on NC carried out in Santa Catarina state and its continuity can play an important role in the diagnosis and epidemiology of the disease. The association of neuroimages suggestive of NC with a history of epilepsy in our region led us to suggest the need to introduce the search for NC in the patients suffering from epilepsy, by means of a trustworthy and available diagnostic methods in the laboratory routine.

RESUMO

Sorodiagnóstico da neurocisticercose em pacientes com crises epiléticas, por meio de ELISA e immunoblot

Amostras de soro de 88 pacientes dos Estados de Santa Catarina e São Paulo, Brasil, com crises epilépticas e que se submeteram a exame de Tomografia Computadorizada (TC), foram examinadas para detecção de anticorpos anti-cisticercos de *Taenia solium* por meio de ELISA e Immunoblot (IB) utilizando-se os seguintes antígenos: extrato salino total de cisticercos de *T. solium* (Tso); líquido vesicular de *Taenia crassiceps* (Tcra-vf) e glicoproteínas purificadas de cisticercos de *T. crassiceps* (Tcra-gp). Os resultados de ELISA com os antígenos Tso, Tcra-vf e Tcra-gp mostraram 95%, 90% e 80% de sensibilidade, respectivamente, e 68%, 85% e 93% de especificidade, respectivamente. No grupo de pacientes epilépticos, a positividade do ELISA foi 30%, 51% e 35% com os antígenos Tso, Tcra-vf e Tcra-gp, respectivamente. Considerando o IB como teste confirmatório, a positividade foi de 16% (14/88) no grupo total de pacientes epilépticos e 22% (12/54) no grupo de pacientes epilépticos com TC positiva e sinais clínicos compatíveis com neurocisticercose. Foi encontrada correlação estatística significativa entre os resultados de ELISA ou IB e a fase da doença com quaisquer dos antígenos utilizados ($p < 0,05$). Os resultados indicam a necessidade de introduzir na rotina dos laboratórios o diagnóstico de neurocisticercose nos pacientes com convulsões epilépticas devido ao elevado risco de aquisição da cisticercose em nossa região e sua participação na etiologia da epilepsia.

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