Evaluation of cysticercus-specific IgG (total and subclasses) and IgE antibody responses in cerebrospinal fluid samples from patients with neurocysticercosis showing intrathecal production of specific IgG antibodies

Avaliação das respostas de anticorpos anti-cisticercos IgG (total e subclasses) e IgE em amostras de líquido cefalorraquidiano de pacientes com neurocisticercose apresentando produção intratecal de anticorpos específicos IgG

Lisandra Akemi Suzuki, Cláudio Lúcio Rossi

ABSTRACT

In the present study, an enzyme-linked immunosorbent assay (ELISA) standardized with vesicular fluid of *Taenia solium* cysticerci was used to screen for IgG (total and subclasses) and IgE antibodies in cerebrospinal fluid (CSF) samples from patients with neurocysticercosis showing intrathecal production of specific IgG antibodies and patients with other neurological disorders. The following results were obtained: IgG-ELISA: 100% sensitivity (median of the ELISA absorbances (MEA)=1.17) and 100% specificity; IgG_1-ELISA: 72.7% sensitivity (MEA=0.49) and 100% specificity; IgG_2-ELISA: 81.8% sensitivity (MEA=0.46) and 100% specificity; IgG_3-ELISA: 63.6% sensitivity (MEA=0.12) and 100% specificity; IgG_4-ELISA: 90.9% sensitivity (MEA=0.85) and 100% specificity; IgE-ELISA 93.8% sensitivity (MEA=0.60) and 100% specificity. There were no significant differences between the sensitivities and specificities in the detection of IgG-ELISA and IgE-ELISA, although in CSF samples from patients with neurocysticercosis the MEA of the IgG-ELISA was significantly higher than that of the IgE-ELISA. The sensitivity and MEA values of the IgG_4-ELISA were higher than the corresponding values for the other IgG subclasses. Future studies should address the contribution of IgG_4 and IgE antibodies to the physiopathology of neurocysticercosis.

Key words: IgG, IgE, enzyme-linked immunosorbent assay, neurocysticercosis.

RESUMO

No presente estudo, uma reação imunoenzimática (ELISA) padronizada com o fluido vesicular de cisticercos de *Taenia solium* foi utilizada para avaliar as respostas de anticorpos anti-cisticercos IgG (total e subclasses) e IgE em amostras de líquido cefalorraquidiano (LCR) de pacientes com neurocisticercose apresentando produção intratecal de anticorpos específicos IgG e pacientes com outras desordens neurológicas. Os seguintes resultados foram obtidos: ELISA-IgG: 100% de sensibilidade (mediana das absorbâncias das reações ELISA (MAE)=1,17) e especificidade 100%; ELISA-IgG_1: sensibilidade 72,7% (MAE=0,49) e especificidade 100%; ELISA-IgG_2: sensibilidade 81,8% (MAE=0,46) e especificidade 100%; ELISA-IgG_3: sensibilidade 63,6% (MAE=0,12) e especificidade 100%; ELISA-IgG_4: sensibilidade 90,9% (MAE=0,85) e especificidade 100%; ELISA-IgE: sensibilidade 93,8% (MAE=0,60) e especificidade 100%. Não foram encontradas diferenças significativas entre as sensibilidades e especificidades das reações ELISA-IgG e ELISA-IgE, embora a MAE da reação ELISA-IgG em amostras de LCR de pacientes com neurocisticercose tenha sido significativamente maior que a obtida com ELISA-IgE. Os valores de sensibilidade e MAE da reação ELISA-IgG_4 foram maiores que os valores correspondentes para as outras subclasses da IgG. Estudos futuros deverão abordar a contribuição dos anticorpos IgG_4 e IgE na fisiopatologia da neurocisticercose.

Palavras-Chave: IgG, IgE, ensaio de imunoadsorção enzimática, neurocisticercose.

Department of Clinical Pathology, Faculty of Medical Sciences, State University of Campinas (UNICAMP), Campinas SP, Brazil.

Correspondence: Cláudio Lúcio Rossi; Departamento de Patologia Clínica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas (UNICAMP); Rua Tessália Vieira de Camargo 126; 13083-887 Campinas SP - Brasil; E-mail: clr@fcm.unicamp.br

Support: Research supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Brazil.

Conflict of interest: There is no conflict of interest to declare.

Received 14 June 2012; Received in final form 17 September 2012; Accepted 24 September 2012

Human neurocysticercosis, a severe disease caused by the presence of *Taenia solium* cysticerci in the central nervous system (CNS), is an important public health problem in many developing countries, including Brazil[1-4]. The clinical manifestations of neurocysticercosis are variable and non-specific, and depend upon the cyst load, the topographic localization of cysts, the parasite's biological state and the host's immune response[5-8]. Neuroimaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) are recognized as the gold standard for the diagnosis of neurocysticercosis[2]. The detection of specific antibodies against *T. solium* cysticercal antigens in cerebrospinal fluid (CSF) samples by enzyme-linked immunosorbent assay (ELISA) has been considered a useful diagnostic element for neurocysticercosis, especially when neuroimaging techniques are unavailable or inconclusive[9,10]. Besides their importance in the diagnosis of neurocysticercosis, neuroimaging findings and the detection of specific antibodies in CSF samples have contributed to a better understanding of the physiopathological processes of this infection.

Few reports in the literature have assessed the simultaneous production of different classes and subclasses of cysticercus-specific antibodies in patients with neurocysticercosis. The purpose of the present study was to evaluate cysticercus-specific IgG (total and subclasses) and IgE antibody responses in CSF samples from patients with neurocysticercosis showing intrathecal production of specific IgG antibodies.

METHODS

CSF samples

A collection of 56 CSF samples was screened for the presence of cysticercus-specific IgG (total and subclasses) by means of ELISA. The samples were obtained from 22 patients with neurocysticercosis showing intrathecal production of specific IgG antibodies and 34 patients with other neurological disorders (neurosyphilis [n=5], cryptococcal meningitis [n=8], toxoplasmosis [n=4], multiple sclerosis [n=5], viral meningitis [n=6] and bacterial meningitis [n=6]). In six CSF samples from patients with neurocysticercosis and three CSF samples from patients with other neurological disorders (one cryptococcal meningitis, one multiple sclerosis and one viral meningitis), the volume of material available was insufficient for the screening of IgE antibodies. CSF samples from patients with neurocysticercosis and patients with other neurological disorders were used to evaluate the sensitivity and specificity of the assays, respectively. The patients with neurocysticercosis had CT and/or MRI results compatible with this disease, whereas the patients with other neurological disorders had no clinical and epidemiological evidence of infection by *T. solium*. All of the patients with neurocysticercosis included in this study had intrathecal synthesis of cysticercus-specific IgG antibodies. The detection of intrathecal antibody production was based on calculation of the specific IgG antibody index, as previously described[11]. All of the patients were attended at the University Hospital of the State University of Campinas (UNICAMP). This study was approved by the Ethics Committee of the School of Medical Sciences, UNICAMP, in accordance with the resolutions of the Brazilian National Ethics Committee.

Antigen preparation

Vesicular fluid (VF) from *T. solium* cysticerci was used as the antigen preparation in the ELISAs. The VF was obtained as previously described[12]. Briefly, *T. solium* cysticerci were extracted from tissues from a naturally infected pig using a scalpel. Calcified parasites and those in the process of degeneration were discarded. After several washes with 0.15 M sterile phosphate-buffered saline (PBS), pH 7.2, the parasites were ruptured individually using two needles and the VF was collected with a Pasteur pipette and transferred to centrifuge tubes. The material was centrifuged at 10,000 g for 30 minutes at 4ºC, and the supernatant was sonicated for 1 minute (30 s sonication/30 s pause) in an ice-water bath using a Branson Sonicator (model SX 30) at a power setting of 3 with a 20% pulse duty cycle. After sonication, enzyme inhibitors (phenylmethylsulfonyl fluoride (PMSF) and leupeptin, final concentrations of 5 mM and 0.0025 mM, respectively) were added to the solution, the protein concentration was determined[13] and aliquots were stored at -80ºC.

ELISA for detection of total IgG

The assay was done as previously described[14]. Briefly, the antigen preparation was diluted to 4 µg of protein/mL in 0.1 M carbonate-bicarbonate buffer, pH 9.5, prior to sensitizing the wells of U-bottomed ELISA plates. After antigen sensitizing, the wells were washed once with 200 µl of PBS containing 0.1% Tween 20 (PBS-T) and the reactive sites in the polystyrene wells were blocked with 100 µl of PBS-T containing 0.1% bovine serum albumin. After incubation for 30 minutes at room temperature (RT) and washing twice with PBS-T, 100 µL of CSF samples diluted 1:5 in PBT-T was added to the wells. Following an one hour incubation at RT, the wells were washed three times with PBS-T, and 100 µl of the conjugate (diluted 1:800 in PBS-T) was added to each well. After incubation for one hour at RT and three washes with PBS-T, 100 µl of the substrate system [tetramethylbenzidine (TMB)/H_2O_2] was added to the wells. Ten minutes after substrate addition the reactions were stopped by adding 50 µl of 2 N H_2SO_4 to each well, and the resulting absorbances were read at 450 nm in an ELISA reader. Positive and negative controls were included in each plate. Each CSF sample was assayed in duplicate and the mean absorbance determined. The final absorbance for each CSF sample was determined by subtracting the mean absorbance of the two antigen controls in the corresponding plate. The cut-off value for the assay was determined using a ROC (Receiver Operating Characteristic) curve[15].

ELISA for detection of IgG subclasses and IgE

All reagents were obtained from Sigma-Aldrich Corporation (Saint Louis, Missouri, USA) unless otherwise specified. The assays were done as described above for the detection of total IgG, with the following modifications. The CSF samples were diluted 1/5 for IgG subclasses and 1/2 for IgE (for IgE detection, the samples were pre-absorbed with RF Absorbent, Siemens, Marburg, Germany). After incubation with the CSF samples and washing with PBS-T, 100 µl of mouse monoclonal antibody specific for one of the four subclasses (anti-IgG_1, anti-IgG_2, anti-IgG_3 or anti-IgG_4, diluted 1/750 in PBST-T) and IgE (diluted 1/1000 in PBS-T) was added to the wells. After incubation for one hour at RT, the wells were washed three times with PBS-T, and 100 µl of conjugate (peroxidase conjugated sheep anti-mouse IgG diluted 1/1000 in PBS-T) was added to the wells. After further incubation for one hour at RT, the wells were washed three times with PBS-T, and 100 µl of the substrate system (TMB/H_2O_2) was added to each well. The cut-off values for the assays were determined using a ROC curve[15].

Data analysis

The sensitivities of the IgG-ELISA and IgE-ELISA and the medians of the ELISA absorbances in CSF samples from patients with neurocysticercosis were compared using the McNemar and Wilcoxon tests, respectively[16]. The sensitivities of the ELISAs for the IgG subclasses and the medians of the ELISA absorbances in CSF samples from patients with neurocysticercosis were compared using the Cochran and Friedman tests, respectively[16]. Differences among the results were considered significant when $p \leq 0.05$.

RESULTS

The sensitivities and specificities of the ELISAs for the detection of IgG and its subclasses and IgE, as well as the median of the ELISA absorbances (MEA) in CSF samples from patients with neurocysticercosis, are shown in Table. There were no significant differences between the sensitivities and specificities for the detection of IgG-ELISA and IgE-ELISA, although the MEA of the IgG-ELISA in CSF samples from patients with neurocysticercosis was significantly higher than that of the IgE-ELISA. The sensitivity and MEA values of the IgG_4-ELISA were higher than the corresponding values for the other IgG subclasses.

Table. ELISA results for the detection of cysticercus-specific antibodies.

Reaction	Sensitivity (%) (MEA)	Specificity (%)
IgG	100 (1.17)	100
IgG_1	72.7 (0.49)	100
IgG_2	81.8 (0.46)	100
IgG_3	63.6 (0.12)	100
IgG_4	90.9 (0.85)	100
IgE	93.8 (0.60)	100

ELISA: enzyme-linked immunosorbent assay; MEA: median of the ELISA absorbances.

DISCUSSION

Few studies have examined the simultaneous detection of cysticercus-specific total IgG and IgE in CSF samples from patients with neurocysticercosis[17-19]. In these studies, based on ELISAs standardized with crude cysticercal extracts, the sensitivities of the reactions for detecting IgG and IgE antibodies ranged from 11.8 to 100% and from 0 to 31.8%, respectively. There is also only limited data on the detection of IgG subclasses and IgE in CSF. Short et al.[20] used a quantitative radioimmunoassay to detect significantly elevated levels of specific IgG_4 (86 arbitrary units [AU]/mL) in CSF samples from patients with neurocysticercosis compared to the control group (1.6 AU/mL), whereas no specific IgE antibody response was detected in the CSF samples tested. Chavarría et al.[21] found that the severity of infection was related to increased cellularity in the CSF, which was, in turn, characterized by increased levels of all IgG subclasses but not of IgE.

Increased production of IgG_4 and IgE antibodies has been reported in helminth infections[22]. IgG_4 is generally considered a non-pathogenic antibody because of its inefficiency in activating the complement system and in forming large immune complexes, which could result in serious problems in chronic antigen stimulation[23]. The induction of IgG_4 antibodies is believed to be a major mechanism used by parasites to evade the host's immune system[24]. Many months of repeated exposure to antigen are usually required before the IgG_4 response becomes prominent[23]. Infection with larval *T. solium* represents a chronic exposure and may account for the high levels of specific IgG_4 found in patients with neurocysticercosis[20].

In the present study, IgG_4 and IgE antibodies were detected in 90.9 and 93.8% of the CSF samples from patients with neurocysticercosis. This detection was based on an ELISA standardized with VF from *T. solium* cysticerci. VF appears to be suitable for antibody detection by ELISA in neurocysticercosis when compared with other cysticercal antigens[12,14,18,25-27].

There was a marked discrepancy between the frequency of anti-cysticercal IgE antibodies detected here in CSF from patients with neurocysticercosis and that reported in the literature[17-19]. Variations in the immune response in neurocysticercosis are expected and are probably related to several factors, including heterogeneity of the patients included in the study, the immune status of the patients at the time of blood and/or CSF sample collection, the number, location and evolutionary stage of the parasites in the CNS, the intrinsic properties of the techniques used for antibody

detection, the antigen preparation and the method of calculating the cut-off of the reactions. Two aspects of our work deserve to be highlighted, namely, (1) that to avoid false-negative results in the IgE-ELISA all CSF samples from patients with neurocysticercosis were pre-absorbed with RF Absorbent and (2) that all patients with neurocysticercosis had intrathecal production of anti-cysticercus IgG antibodies. The diagnosis of neurocysticercosis frequently depends on a combination of clinical, epidemiological, neuroimaging and laboratory data. In the present study, all patients with neurocysticercosis had neuroimaging findings compatible with neurocysticercosis and specific IgG antibody index indicative of intrathecal antibody production. The demonstration of intrathecal antibody production has proven useful for showing the involvement of the CNS in several diseases, including cysticercosis[11,28]. The detection of intrathecal antibody synthesis allows the immunologic diagnosis of the disease even when inflammatory alterations are not found in the CSF[28].

The marked IgG_4 and IgE responses seen here in CSF samples from patients with neurocysticercosis merit further investigation. Future studies should address the contribution of these antibodies to the physiopathology of neurocysticercosis.

References

1. Spina-França A, Livramento JA, Machado LR. Cysticercosis of the central nervous system and cerebrospinal fluid. Immunodiagnosis of 1573 patients in 63 years (1929-1992). Arq Neuropsiquiatr 1993;51:16-20.
2. Sciutto E, Fragoso G, Fleury A, et al. Taenia solium disease in humans and pigs: an ancient parasitosis disease rooted in developing countries and emerging as a major health problem of global dimensions. Microbes Infect 2000;2:1875-1890.
3. Carpio A. Neurocysticercosis: an update. Lancet Infect Dis 2002;2:751-762.
4. Agapejev S. Aspectos clínico-epidemiológicos da neurocisticercose no Brasil: uma abordagem crítica. Arq Neuropsiquiatr 2003;61: 822-828.
5. Sotelo J, Guerrero V, Rubio F. Neurocysticercosis: a new classification based on active and inactive forms. A study of 753 cases. Arch Intern Med 1985;145:442-445.
6. Del Brutto OH, Sotelo J. Neurocysticercosis: an update. Rev Infect Dis 1988;10:1075-1087.
7. Pittella JEH. Neurocysticercosis. Brain Pathol 1997;7:681-693.
8. Takayanagui OM, Odashima NS. Clinical aspects of neurocysticercosis. Parasitol Int. 2006;55:S111-S115.
9. Del Brutto OH, Rajshekhar V, White Jr AC, et al. Proposed diagnostic criteria for neurocysticercosis. Neurology 2001;57:177-183.
10. Garg RK. Diagnostic criteria for neurocysticercosis: some modifications are needed for Indian patients. Neurol India 2004;52:171-177.
11. Arruda GC, Quagliato EM, Rossi CL. Intrathecal synthesis of specific immunoglobulin G antibodies in neurocysticercosis: evaluation of antibody concentrations by enzyme-linked immunosorbent assay using a whole cysticercal extract and cyst vesicular fluid as antigens. Diagn Microbiol Infect Dis 2006;54:45-49.
12. Suzuki LA, Arruda GC, Quagliato EM, Rossi CL. Evaluation of Taenia solium and Taenia crassiceps cysticercal antigens for immunodiagnosis of neurocysticercosis using ELISA on cerebrospinal fluid samples. Rev Soc Bras Med Trop 2007;40:152-155.
13. Bradford MM. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-254.
14. Suzuki LA, Rossi CL. Evaluation of two Taenia solium cysticercal antigenic preparations (vesicular fluid and a glycoprotein fraction with affinity for lentil lectin) for the immunodiagnosis of neurocysticercosis by enzyme-linked immunosorbent assay. Arq Neuropsiquiatr 2011;69:470-474.
15. Fletcher R, Fletcher S. Clinical epidemiology: the essentials. 2005. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2005.
16. Conover WJ. Practical nonparametric statistics. 2nd ed. New York: John Wiley & Sons Inc.; 1971.
17. Espinoza B, Ruiz-Palacios G, Tovar A, Sandoval MA, Plancarte A, Flisser A. Characterization by enzyme-linked immunosorbent assay of the humoral immune response in patients with neurocysticercosis and its application in immunodiagnosis. J Clin Microbiol 1986;24:536-541.
18. Bueno EC, Vaz AJ, Machado LR, Livramento JA. Detection of IgG, IgA and IgE antibodies in cerebrospinal fluid, serum and saliva samples by ELISA with Taenia solium and Taenia crassiceps antigens. Arq Neuropsiquiatr 2000;58:18-24.
19. Odashima NS, Takayanagui OM, Figueiredo JF. Enzyme-linked immunosorbent assay (ELISA) for the detection of IgG, IgM, IgE and IgA against Cysticercus cellulosae in cerebrospinal fluid of patients with neurocysticercosis. Arq Neuropsiquiatr 2002;60:400-405.
20. Short JA, Heiner DC, Hsiao RL, Andersen FL. Immunoglobulin E and G_4 antibodies in cysticercosis. J Clin Microbiol 1990;28:1635-1639.
21. Chavarría A, Fleury A, García E, Márquez C, Fragoso G, Sciutto E. Relationship between the clinical heterogeneity of neurocysticercosis and the immune-inflammatory profiles. Clin Immunol 2005;116:271-278
22. Garraud O, Perraut R, Riveau G, Nutman TB. Class and subclass selection in parasite-specific antibody responses. Trends Parasitol 2003;19:300-304.
23. Aalberse RC, Stapel SO, Schuurman J, Rispens T. Immunoglobulin G_4: an odd antibody. Clin Exp Allergy 2009;39:469-477.
24. Adjobimey T, Hoerauf A. Induction of immunoglobulin G4 in human filariasis: an indicator of immunoregulation. Ann Trop Med Parasitol 2010;104:455-464.
25. Larralde C, Sotelo J, Montoya RM, et al. Immunodiagnosis of human cysticercosis in cerebrospinal fluid. Antigens from murine Taenia crassiceps cysticerci effectively substitute those from porcine Taenia solium. Arch Pathol Lab Med 1990;114:926-928.
26. Bueno EC, Snege M, Vaz AJ, Leser PG. Serodiagnosis of human cysticercosis by using antigens from vesicular fluid of Taenia crassiceps cysticerci. Clin Diag Lab Immunol 2001;8:1140-1144.
27. Arruda GC, Silva AD, Quagliato EM, Maretti MA, Rossi CL. Evaluation of Taenia solium and Taenia crassiceps cysticercal antigens for the serodiagnosis of neurocysticercosis. Trop Med Int Health 2005;10:1005-1012.
28. Machado LR, Livramento JA, Vaz AJ, et al. IgG intrathecal synthesis and specific antibody index in patients with neurocysticercosis. Arq Neuropsiquiatr 2002:60:395-399.