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BRIEF COMMUNICATION

A RAPID LATEX AGGLUTINATION TEST FOR THE DETECTION OF ANTI-CYSTICERCUS ANTIBODIES IN CEREBROSPINAL FLUID (CSF)

Sérgio M. ROCHA(1), Lisandra A. SUZUKI(1), Andréa D.T. da SILVA(1), Gisele C. ARRUDA(1,2) & Cláudio L. ROSSI(1)

SUMMARY

Simple and rapid latex-based diagnostic tests have been used for detecting specific antigens or antibodies in several diseases. In this article, we present the preliminary results obtained with a latex agglutination test (LAT) for diagnosing neurocysticercosis by detection of antibodies in CSF. A total of 43 CSF samples were assayed by the LAT: 19 CSF samples from patients with neurocysticercosis and 24 CSF samples from patients with other neurologic disorders (neurosyphilis, n = 8; neurotoxoplasmosis, n = 3; viral meningitis, n = 4, chronic headache, n = 9). The LAT exhibited 89.5% sensitivity and 75% specificity. The use of LAT seems to be an additional approach for the screening of neurocysticercosis with advantage of simplicity and rapidity. Further studies could be performed using purified antigens and serum samples.

KEYWORDS: *Taenia solium* cysticercus; Latex agglutination test; Antibody detection.

Cysticercosis is an important health problem in many countries of Asia, Africa and Latin America with inadequate sanitary conditions[1,6]. The disease is caused by infection with the larval form (cysticercus) of the pork tapeworm *Taenia solium*. The cysticerci may be located in areas where they produce no symptoms, such as muscle or cutaneous tissues. On the other hand, the presence of cysticerci in the central nervous system (CNS), a condition known as neurocysticercosis, can cause seizures and other neurologic problems. The clinical manifestations of neurocysticercosis are nonspecific and varied and depend on the number, size, age, localization and evolutionary stage of cysticerci in the SNC[6,7]. Thus, the definitive diagnosis of neurocysticercosis should always be considered in an epidemiological context and confirmed by neuro-imaging techniques[3] (computed tomography or nuclear magnetic ressonance) and/or detection of specific antibodies in cerebrospinal fluid (CSF)[4-5]. Several tests have been used for the immunodiagnosis of neurocysticercosis such as complement fixation (CFT), immunoelectrophoresis (IEE), indirect hemagglutination (IHT), indirect immunofluorescence (IFT), enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immunoelectrotransfer blot (EIBT) assay[4,5,7]. The performance of immunoenzymatic techniques is superior to other techniques for the immunodiagnosis of neurocysticercosis. However, these techniques require several laboratory equipments and highly skilled personnel. Hence, development of simple, rapid and sensitive methods for detecting antibodies against *T. solium* cysticerci are needed. Simple and rapid latex-based diagnostic tests have been used for detecting specific antigens or antibodies in several diseases[2]. In this article, we present the preliminary results obtained with a latex agglutination test (LAT) for diagnosing neurocysticercosis by detection of antibodies in CSF.

All chemicals were reagent grade or better and, unless otherwise stated, were obtained from Sigma Chemical Co., St. Louis, Missouri, USA.

The *Cysticercus* antigen was prepared as previously described[4], with a few modifications. Briefly, frozen cysts were quickly thawed and resuspended in phosphate buffered saline (PBS) 0.15 M pH 7.2 containing protease inhibitors [5 mM phenylmethylsulfonyl fluoride (PMSF) and 0.0025 mM leupeptin] to about three times the cysticerci volume and the material was homogeneized in an ice-water bath using a Polytron® homogeneizer (Brinkmann Instruments, Inc., Westbury, New York, USA) equipped with a PT-20 ST probe. Homogeneization was accomplished in three 30-sec pulses with the probe speed set at 3 and the pulses separated by a 30-sec pause for sample cooling. The homogenate was sonicated in an ice-water bath using a Branson Sonicator (model SX-30, Branson Ultrasonics, Danbury, USA). Sonication was accomplished with a 20% pulse dute in three 1-min pulses with the probe speed set at 3 and the pulses separated by a 1 min pause for sample cooling. Protease inhibitors PMFS and leupetin were added to sonicated material as previously described and the suspension was gently stirred for 16 hours at 5 °C. The material was centrifuged at 20,000 g for 1 h at 4 °C. The supernatant was carefully removed, filtered through 0.45 µm filters (Millex filters, Millipore Corporation, USA), and was stored in aliquots at – 80 °C until used.

(1) Departamento de Patologia Clínica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, São Paulo, Brasil.
(2) Bolsista FAPESP. São Paulo, SP, Brasil.
Correspondence to: Cláudio Lúcio Rossi, Departamento de Patologia Clínica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, C. P. 6111, 13083-970 Campinas, São Paulo, Brasil.

Carboxyl-modified polystyrene latex microspheres (0.77 μm diameter, 100 mg/ml, Bangs Laboratories, Fishers, USA) were used in the agglutination test. After homogeneization, 0.5 ml of the latex suspension was washed with MES [2-(n-morpholino)-ethanesulfonic acid] buffer, pH 5.5 (activation buffer), twice by centrifugation at 13,000 rpm for 10 min each time, and the microspheres were ressuspended in same buffer to 10 mg/ml. For covalent coupling of *Cysticercus* antigen to microspheres, 50 mg of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide freshly dissolved in 0.5 ml of water were added to latex suspension and the mixture was gently stirred for 15 min. The material was then washed with MES buffer, pH 7.2 (coupling buffer) as previously described and the microspheres were resuspended in the same buffer to 2.5 ml. The material was transfered to a becker and mixed with an equal volume of *Cysticercus* antigen diluted to 1 mg/ml with the coupling buffer. The mixture was incubated at room temperature for 3 h with constant shaking and then 1 ml of a 10% solution of bovine serum albumin (BSA) in coupling buffer was added. After an incubation of 30 min, the microspheres were washed with PBS 0.15 M pH 7.4 containing 0.1% BSA and the latex particles were resuspended in the same buffer to 10 mg/ml. The sensitized latex particles were stored at 4 °C until use.

A total of 43 CSF samples were assayed by the LAT: 19 CSF samples from patients with neurocysticercosis and 24 CSF samples from patients with other neurologic disorders (neurosyphilis, n = 8; neurotoxoplasmosis, n = 3; viral meningitis, n = 4, chronic headache, n = 9). All 19 patients with neurocysticercosis had evidence of cystic lesions on computed tomography. All 24 patients with other neurological disorders had no significant epidemiologic, clinical or neuro-imaging data for neurocysticercosis.

The LAT was performed by mixing 40 μl of the sensitized latex with 40 μl of the CSF sample on a glass slide. Positive and negative controls were included in each assay. The slide was gently rotated and rocked for 2 min, and the reactions were visually observed. Reactions presenting a clear agglutination of latex particles were recorded as positive.

The LAT results of all the CSF tested are shown in the Table 1. Positive reactions were detected in 17 of 19 patients with neurocysticercosis and in 6 of 24 patients with other neurological disorders. Four neurosyphilis CSF samples and two neurotoxoplasmosis CSF samples had positive LAT results. Based on these results the LAT exhibited 89.5% sensitivity and 75% specificity.

The detection of specific antibodies against *T. solium* cysticerci antigens in CSF from patients suspected of having neurocysticercosis is an additional tool for the diagnosis. However, when the immunological tests are performed with a whole cysticerci extract, there are a significant number of false-positive results because the cross-reactivity with other parasitic diseases, including syphilis and toxoplasmosis[4].

Table 1
LAT results in CSF samples from patients with different neurological disorders

Diagnosis	Agglutination reactions	
	Positive	Negative
Neurocysticercosis (n = 19)	17	2
Neurosyphilis (n = 8)	4	4
Neurotoxoplasmosis (n = 3)	2	1
Viral meningitis (n = 4)	0	4
Chronic cefalea (n = 9)	0	9

Numerous latex-based assays have found commercial applications. Advantages include assay speed, simplicity, low cost of manufacture and lack of a requirement for sophisticated laboratory equipments[2]. The use of LAT seems to be an additional approach for the screening of neurocysticercosis with advantage of simplicity and rapidity. Further studies could be performed using purified antigens and serum samples.

RESUMO

Teste rápido de aglutinação utilizando partículas de látex para a detecção de anticorpos anti-cisticercos em amostras de líquido cefalorraquidiano (LCR)

Testes diagnósticos simples e rápidos baseados na aglutinação de partículas de látex têm sido utilizados para a pequisa de antígenos ou anticorpos específicos em muitas doenças. No presente trabalho, é descrito um teste de aglutinação em lâmina para a pesquisa de anticorpos contra cisticercos de *Taenia solium*, utilizando partículas de látex revestidas com um extrato bruto do parasita. Anticorpos anti-cisticercos foram pesquisados em 19 amostras de LCR de pacientes com neurocisticercose e em 24 amostras de LCR de pacientes com outros problemas neurológicos (neurosífilis, n = 8; neurotoxoplasmose, n = 3; meningite viral, n = 4; cefaléia crônica, n = 9). O teste de aglutinação apresentou sensibilidade e especificidade de 89,5% e 75%, respectivamente. O teste de aglutinação para cisticercose idealizado é simples, rápido e barato. Essas características tornam o teste um meio promissor de expansão e simplificação do imunodiagnósico da neurocisticercose. Estudos futuros poderiam testar a sensibilização das partículas de látex com antígenos de cisticercos purificados e a pesquisa de anticorpos em amostras de soros.

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