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Evaluation of intrathecal synthesis of specific IgG antibodies against *Toxoplasma gon*dii in the diagnosis assessment of presumptive *toxoplasma* encephalitis in aids patients

Avaliação da síntese intratecal de anticorpos IgG anti-*Toxoplasma gondii* para o diagnóstico da neurotoxoplasmose em pacientes com síndrome da imunodeficiência adquirida

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ABSTRACT

*The diagnosis of neurotoxoplasmosis in patients with acquired immunodeficiency syndrome is mainly based on tomographic or magnetic resonance findings and on the response to specific treatment. We studied 55 patients with AIDS and neurotoxoplasmosis according to these diagnostic criteria (group 1), 37 patients with AIDS and neurological involvement of other etiology (group 2), and 16 anti-HIV-negative individuals with neurological manifestations (group 3). Serum and cerebrospinal fluid were examined for the presence of anti-*T. gondii *IgG, by indirect immunofluorescence. In 72 of them, the total amounts of these antibodies were determined in order to assess local production of anti-*T. gondii *antibodies in the central nervous system and to correlate their titers with infection activity in patients with AIDS and neurotoxoplasmosis. IgG titers ≥ 1/64 in cerebrospinal fluid reached 100% specificity for the diagnosis of neurotoxoplasmosis in AIDS. Evidence of local synthesis of these antibodies was detected in 42.8% of patients of group 1, in 29.1% of patients of group 2 and in no patient of group 3. The test showed 70.8% specificity and therefore was not useful in our study for the differential diagnosis of neurotoxoplasmosis in patients with AIDS.*

Key-words: *AIDS. Neurotoxoplasmosis. Antibody synthesis. Central nervous system.*

RESUMO

*O diagnóstico da neurotoxoplasmose em pacientes com síndrome da imunodeficiência adquirida baseia-se fundamentalmente nos achados tomográficos ou de ressonância magnética e na resposta ao tratamento específico. Estudamos 55 pacientes com SIDA e neurotoxoplasmose, de acordo com estes critérios diagnósticos (grupo 1); 37 pacientes com SIDA e comprometimento neurológico por outra etiologia (grupo 2) e 16 indivíduos anti-HIV negativo, com outras doenças neurológicas (grupo 3), pesquisando IgG, anti-*T. gondii*, no soro e no líquor, utilizando a reação de imunofluorescência indireta. Em 72 casos, determinamos os teores totais destes anticorpos aí presentes, com objetivo de avaliar a produção local, no sistema nervoso central, de anticorpos específicos e correlacionar os títulos com atividade da infecção, em pacientes com SIDA e neurotoxoplasmose. Evidência de produção local destes anticorpos foi detectada em 42,8% dos pacientes do grupo 1, em 29,1% dos pacientes do grupo 2 e em nenhum paciente do grupo 3. O teste apresentou especificidade intermediária (70,8%), porém não foi útil para o diagnóstico diferencial da neurotoxoplasmose em pacientes com SIDA, em nosso estudo. Por outro lado, títulos de IgG no líquor ≥1/64 alcançaram 100% de especificidade para o diagnóstico de neurotoxoplasmose na SIDA.*

Palavras-chaves: *SIDA. Neurotoxoplasmose. Síntese de anticorpos. Sistema Nerevoso Central.*

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Neurotoxoplasmosis is one of the most frequent opportunistic infections compromising the central nervous system (CNS) of patients with acquired immunodeficiency syndrome (AIDS), with a prevalence of 3% to 50% and high morbidity and mortality if not diagnosed and treated early[9 12].

In most cases the disease results from reactivation of a latent infection, but cases of acute infection with dissemination have been reported[2 15]. The estimated risk of toxoplasmosis reactivation in the CNS of patients with AIDS who present anti-*Toxoplasma gondii* IgG antibodies in serum ranges from 12 to 47%[13 14 30].

The clinical presentation is variable, manifesting as diffuse encephalitis, meningoencephalitis or, more commonly, as a tumoral lesion with a mass effect. Motor syndrome, conscience disorders, seizures and focal signs are common manifestations[16 32], so that this entity is clinically indistinguishable from other CNS complications also frequently occurring in these patients, such as primary CNS lymphoma, viral or fungal encephalitis, reactivation of Chagas' disease, neurotuberculosis, and others[11 23 33].

An early diagnosis of neurotoxoplasmosis in these cases has been limited by the lack of more sensitive and specific noninvasive methods. Cerebrospinal fluid findings are nonspecific, varying from normality to mild lymphocytic pleocytosis, with normal or only slightly elevated spinal fluid protein levels. Imaging studies such as computed tomography (CT) of the skull and magnetic resonance (MR) are of high diagnostic value by revealing isodense or hypodense single or multiple lesions with a mass effect that take up contrast in an annular or nodular form. These findings are detected in about 90% of cases and are highly suggestive of neurotoxoplasmosis, although not pathognomonic[24 35].

The serologic profile of these patients is similar to that of the general population with chronic infection. IgM antibodies are not commonly detected and IgG antibodies do not discriminate between latent and active infection[14 23 36], and may be undetectable in a minority of cases[13 29 39]. In addition, the activity of the disease does not seem to correlate with IgG antibody titers in serum[18 24 27 33].

In contrast to the reported authors, Hellerbrand et al[17], in a series of 80 cases, observed that high IgG titers in patients with T CD4+ cell numbers < 150 cells/mm^3 indicate the presence of toxoplasmic encephalitis, with a positive predictive value of 88%.

The detection of antibodies in cerebrospinal fluid (CSF) should be interpreted with caution since their presence may simply indicate passive serum antibodies passage to the CSF. However, the demonstration of local synthesis of specific antibodies whose titers increase in the CSF regardless of their increase in serum has proved to be of diagnostic value in some conditions, including neurotoxoplasmosis[26 28 31 34].

We studied 42 patients with AIDS and neurotoxoplasmosis by determining total IgG concentrations in serum and CSF and correlating them with anti-*Toxoplasma gondii* antibody levels in these fluids in order to determine the occurrence of synthesis of specific antibodies at the CNS level and to correlate the titers of these antibodies with infection activity as a subsidy for the diagnosis of neurotoxoplasmosis in AIDS.

MATERIAL AND METHODS

We studied 108 patients, 92 of them infected with human immunodeficiency virus (HIV-1) as determined by 2nd generation ELISA (Abbott), with a diagnosis of AIDS according to CDC (Centers for Disease Control) criteria[5], and 16 non-infected adult patients of both sexes older than 12 years who presented with clinical manifestations of neurological disease were included in the study.

The patients were divided into 3 groups. Group 1 consisted of 55 patients with AIDS and a presumptive diagnosis of neurotoxoplasmosis, group 2 of 37 patients with AIDS, 19 of them with neurocryptococcosis, 3 with neurotuberculosis, 2 with bacterial meningitis, 1 with AIDS-related demential complex, 2 with metabolic alterations affecting the CNS, and 10 with undefined etiology of the neurological signs and symptoms. Group 3 consisted of 18 anti-HIV-negative patients: 7 with bacterial meningitis, 4 with a diagnosis of lymphomonocytic meningitis, 1 with neurocryptococcosis, 1 with CNS paracoccidioidomycosis, 1 with Guillain-Barré syndrome, 1 with subarachnoid hemorrhage, and the last one evaluated for investigation of headache.

The presumptive diagnosis of neurotoxoplasmosis was established based on clinical signs and symptoms, on tomographic findings of single or multiple lesions occupying space with a mass effect and annular or nodular appearance after contrast injection, and on clinical and/or radiologic patient improvement after specific treatment with sulfadiazine (4g/day) and pyrimethamine (50mg/day), or clindamycin (2.7g/day) and pyrimethamine (50mg/day).

The search for anti-*T. gondii* antibodies in serum and CSF was performed by indirect immunofluorescence (IIF) by the method of Camargo[4] using an anti-human IgG fluorescent conjugate (Bio-Meriéux). Serum samples with ≥ 1/16 titers and CSF samples with ≥ 1/1 titers were considered to be positive.

Total IgG concentrations in these fluids were determined in 72 patients by simple radial immunodiffusion by the method of Mancini et al[25], adapted by Barreto[1].

Antibody synthesis at the CNS level was determined using the following index: reciprocal antibody titers in CSF x total IgG concentration in serum/total IgG concentration in CSF x reciprocal antibody titers in serum. Indices with values higher than 1 indicated local antibody synthesis at the CNS level[21 30 33].

Data were analyzed statistically using the Epi Info software, version 6.02. 10/94. The Chi-square test was used, with the level of significance set at $p < 0.05$.

RESULTS

Fifty-four patients (98%) in group 1, 24 (64.8%) patients in group 2 and 10 (62,5%) in group 3 showed evidence of previous *Toxoplasma gondii* infection by the presence of anti-*Toxoplasma gondii* IgG antibodies in serum. Twenty-eight patients in group 1 (51%) had IgG titers ≥ 1/4000,

while in groups 2 and 3 only 13.5% and 5.9% reached these levels, respectively (p = 0.0002) (Table 1).

Anti-*Toxoplasma gondii* IgG antibodies were detected in the CSF of 45/54 (83.4%) group 1 patients, 18/35 (51.4%) group 2 patients and 2/15 (13.3%) group 3 patients. Antibody titers ≥ 1/64 were detected only in group 1 patients (Table 2).

*Table 1 - IgG anti-*Toxoplasma gondii *antibodies in the serum of the three groups of patients studied (IIF).*

Titer	Group 1		Group 2		Group 3	
	nº	%	nº	%	nº	%
Negative	1	1.8	13	35.1	6	35.3
1/16	1	1.8	2	5.4	4	23.5
1/64	4	7.3	5	13.5	4	23.5
1/256	9	16.4	7	19.0	1	5.8
1/1000	12	21.8	5	13.5	-	
1/4000	18	32.7	5	13.5	1	11.7
1/8000	4	7.2	-	-	-	-
1/16000	4	7.2	-	-	-	-
1/32000	2	3.6	-	-	-	-
Total*	55	100.0	37	100.0	16	100.0

* Included patients in whom test for anti-*T. gondii* antibodies in serum was performed

*Table 2 - IgG anti-*Toxoplasma gondii *antibodies in the cerebrospinal fluid of the three patient groups studied (IIF).*

Titer	Group 1		Group 2		Group 3	
	nº	%	nº	%	nº	%
Negative	9	16.6	17	48.5	13	86.7
1/1	3	5.5	1	2.8	1	6.6
1/2	4	7.4	2	5.7	-	
1/4	8	14.8	4	11.4	1	6.6
1/8	8	14.8	6	17.1	-	-
1/16	12	22.2	4	11.4	-	-
1/32	-	-	1	2.8	-	-
1/64	5	9.2	-	-	-	-
1/128	2	3.7	-	-	-	-
1/256	1	1.8	-	-	-	-
1/1000	2	3.7	-	-	-	-
Total*	54	100.0	35	100.0	15	100.0

* Included patients in whom test for anti-*T. gondii* antibodies in the cerebrospinal fluid was performed

Anti-*Toxoplasma gondii* antibody synthesis at the CNS level was detected in 18/42 (42.8%) group 1 patients, in 7/24 (29.1) group 2 patients, and in no patient of group 3 (Table 3).

When we compared group 1 with group 2, calculation of the local rate of antibody synthesis showed low sensitivity (42.8%) and specificity (70.8%), with a positive and negative predictive value of 72% (p = 0.27). The odds ratio was 1.82 (95% CI: 0.56-6.30).

*Table 3 - Index of anti-*Toxoplasma gondii *antibody production in the CNS in the three patient groups studied.*

Titer	Group 1		Group 2		Group 3	
	nº	%	nº	%	nº	%
≤ 1	24	57.2	17	70.8	6	100.0
> 1	18	42.8	7	29.2	0	
Total*	42		24		6	

*Included patients in whom index of anti-*T. gondii* antibody production was performed

Note: when patients in Group 1 were compared with patients in Group 2, the odds ratio was 1.82 (95% CI: 0.56-6.30). *p* = 0.27

DISCUSSION

Most of the expansive lesions of the CNS detected in patients with AIDS are attributed to reactivation of latent *Toxoplasma gondii* infection caused by HIV-induced immunosuppression.

In these cases, the diagnosis of neurotoxoplasmosis has been based on clinical presentation and on tomographic or magnetic resonance findings, which reveal expansive lesions with annular reinforcement, besides an adequate therapeutic response[6 24 34]. Diagnostic confirmation is difficult because clinical signs and symptoms and radiologic imaging findings may be confused with those of other diseases that compromise the CNS of these patients. Thus, the definitive etiologic diagnosis may require invasive and risky procedures.

The detection of specific IgM and IgG class antibodies in serum is a well-established method for the diagnosis of acute primary and congenital *Toxoplasma gondii* infection. The value of this method for immunosuppressed individuals is controversial since in most cases the disease results from reactivation of latent infection occurring during an advanced phase of immune system dysfunction[14 22].

Several serologic tests for the detection of circulating *Toxoplasma gondii* antigens for the detection of specific antibodies have been used in an attempt to establish the diagnosis of neurotoxoplasmosis in patients with AIDS, although with controversial results[7 8 10 20 35 37]. Thus, it would be difficult to establish this diagnosis only on the basis of serum antibody levels since, according to some investigators, the absence of elevated titers would also be insufficient to rule out the occurrence of toxoplasmosis reactivation[17 18 38].

In the present study, the IIF reaction applied to serum for the detection of anti-*Toxoplasma gondii* IgG antibodies showed high sensitivity (S = 98.2%), but reduced specificity (35.1%), and 93% positive predictive value (p < 0.0005). These results, taken together with those obtained in other studies, confirm the idea that the absence of specific IgG antibodies in serum invalidates the diagnosis of toxoplasmosis reactivation[19 34].

When comparing the patient groups studied in terms of serum IgG levels we detected a good correlation between high antibody titers (≥ 1/4000) and a diagnosis of neurotoxoplasmosis, with 85% positive predictive value and 86.5% specificity (p = 0.0002).

Monitoring the kinetics of serum antibody titers could be useful to determine whether these levels increase during the reactivation of disease, not only being very useful for diagnostic purposes, but also permitting the early institution of prophylactic and therapeutic measures.

It has been demonstrated that approximately 50% of patients with *Toxoplasma gondii*-induced encephalitis will present with specific antibodies in the CSF[20 29]. However, this finding should be interpreted with caution since the rupture

of the blood-CSF barrier due to any type of etiology may be accompanied by passive passage of antibodies from serum to CSF. In the present study, the IIF reaction applied to the CSF for the detection of anti-*Toxoplasma gondii* IgG proved to be more specific than serum detection, with a statistically significant difference between groups ($p < 0.005$). Considering the reaction titer, we observed that for titers ≥ 1/64 the test reached 100% specificity ($p = 0.004$), thus representing a good diagnostic marker of neurotoxoplasmosis in AIDS.

Antibody synthesis at the CNS level has been demonstrated in some neurological diseases regardless of alteration of the blood brain barrier responsible for the passage of blood proteins into the CSF [20 25 26 30 37]. Orefice et al[28] detected active antibody synthesis at the CNS level in a series of 4 patients with AIDS and neurotoxoplasmosis compared with 8 patients also with AIDS, but without clinical evidence of neurological disease.

Another study conducted on a larger number of cases including 37 patients with AIDS and toxoplasmic encephalitis, 11 patients with AIDS without neurological disease and 15 individuals with no HIV antibodies but with serologic evidence of *Toxoplasma gondii* infection demonstrated the presence of IgG antibodies in the CSF of 23 (62.2%) of the patients with neurotoxoplasmosis and in none of the patients without neurological disease. Local antibody synthesis was demonstrated in 11 of 16 (68.7%) neurotoxoplasmosis cases and in none of the 4 patients of the control group who showed the presence of antibodies in CSF[29].

In the present study we detected local antibody synthesis in 18/42 (42.8%) patients of the group with neurotoxoplasmosis. Among group 2 and 3 patients, 7/24 (29.1%) and 0/6 showed evidence of anti-*Toxoplasma gondii* antibody synthesis at the CNS level, respectively.

The calculation of the index of local antibody synthesis showed intermediate specificity (70.8%) and low sensitivity (42.8%) for the diagnosis of toxoplasmosis reactivation at the CNS level in AIDS when group 1 patients were compared to group 2 patients, thus being of no use for the differential diagnosis of neurotoxoplasmosis in patients with AIDS. However, there was a statistically significant difference between group 1 and group 3 patients ($p < 0.05$).

We cannot justify these results based only on the advanced immunosuppression of these patients because in many there was no correlation between serum antibody levels and indices of antibody synthesis at the CNS level. Another factor to be considered is that toxoplasmosis is a predominantly parenchymatous disease and that the presence or absence of local antibody production detected by CSF examination may depend on the proximity of the lesion to the meninges.

We observed that 29% of the patients in group 2 presented evidence of production of anti-*Toxoplasma gondii* antibodies in the CNS. These patients were in the same situation of immunosuppression as the patients in group 1 and most (64.8%) demonstrated previous infection with *Toxoplasma gondii*. Therefore, they may have low toxoplasmosis activity in the CNS, with no apparent or incipient clinical or tomographic manifestation since the presence of 2 or more concomitant opportunistic infections is frequently observed in these patients[3 21]. Thus, it would be opportune to evaluate these patients prospectively in order to determine signs of disease reactivation in the future.

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