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IgM AND IgA ANTIBODY RESPONSES IN 12 CASES OF HUMAN ACQUIRED TOXOPLASMOSIS

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SUMMARY

The persistence, in some subjects, of specific IgM antibodies to *Toxoplasma gondii* for several months after the acute phase of infection has complicated the interpretation of serological test results for toxoplasmosis. Several reports have emphasized the value of the detection of *Toxoplasma*-specific IgA antibodies for the diagnosis of acute toxoplasmosis. In this article, we report the follow-up profiles of *Toxoplasma*-specific IgM and IgA antibodies in serum samples obtained from 12 patients at various intervals after the onset of the clinical manifestations of infection. IgM antibodies were detected by the indirect immunofluorescence (IIF) test, antibody capture enzyme-linked immunosorbent assay (cELISA) and enzyme-mediated chemilluminescent technique (CmL). IgA antibodies were quantified by the direct ELISA (dELISA) and cELISA procedures. As defined by the manufacturer of the cELISA test for IgA used, most patients with acute toxoplasmosis have antibody levels > 40 arbritary units per ml (AU/ml). At values > 40 AU/ml, the cELISA for IgA detected significant antibody levels for a shorter time than the other techniques used for IgM and IgA detection. However, IgA levels ≤ 40 AU/ml do not exclude the possibility of acute toxoplasmosis since such levels can be reached very soon after infection with *T. gondii*. The results obtained in the present study show that the serological diagnosis of acute toxoplasmosis may not be such an easy task. Our data suggest that use of the IgA-cELISA concomitantly with IgM antibody screening could permit, in some circumstances, a more efficient diagnosis of acute acquired toxoplasmosis.

KEYWORDS: Toxoplasmosis; Immunodiagnosis; IgM; IgA.

INTRODUCTION

Toxoplasmosis, an infection caused by the intracellular parasite *Toxoplasma gondii*, is generally asymptomatic or is associated with mild, non-specific clinical manifestations in immunocompetent subjects[12, 13]. The diagnosis of toxoplasmosis is usually based on serological tests which detect specific IgM and IgG antibodies. However, several studies have shown that IgM antibodies, classically considered as characteristic of the acute phase of toxoplasmosis, may be detected for many months or even years following the acute phase of infection[4, 8, 10, 16, 21, 25].

Several reports have emphasized the value of the detection of *Toxoplasma*-specific IgA antibodies for the diagnosis of acute human toxoplasmosis[3, 7, 14, 19, 22]. However, considerable controversy still surrounds the persistence of such antibodies following acute infection. Here, we report the follow-up profiles of *Toxoplasma*-specific IgM and IgA antibodies in 12 patients with toxoplasmosis. IgM antibodies were detected by the indirect immunofluorescence (IIF) test, antibody capture enzyme-linked immunosorbent assay (cELISA), and enzyme-mediated chemilluminescent technique (CmL). IgA antibodies were detected by the direct ELISA procedure (dELISA) and by the cELISA test.

MATERIALS AND METHODS

Patients and serum samples

A total of 47 serum samples were obtained from 12 patients with a toxoplasmic infection seen at the University Hospital of the State University of Campinas (Campinas, São Paulo, Brazil). At the time of diagnosis, all 12 patients had clinical manifestations compatible with acute toxoplasmosis; the diagnosis was confirmed by serological tests.

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Serological tests

For the IgM-IIF test, all serum samples were previously treated with rheumatoid factor-absorbent (Behring Company, USA). The IIF test was performed using *T. gondii* tachyzoites as antigen and an anti-human IgM fluorescein conjugate (Biolab Diagnóstica, RJ, Brazil). IgM-IIF antibody titers ≥ 32 were considered positive[8, 27]. The IgM-cELISA was performed using commercial kits (ETI-TOXOK-M) from Sorin Biomedica (Italy). The presence or absence of specific IgM antibodies in each assay was determined by comparing the absorbance values of the unknown samples with those of the cut-off control value. Serum samples with absorbances ≥ the cut-off value were considered positive. The IgM-CmL assay was performed using commercial kits (ACCESS®-TOXO M) from Sanofi Diagnostics Pasteur (France). Values > 140 AU/ml were considered positive. The IgA-dELISA was performed according to TAKAHASHI & ROSSI (1994). Briefly, to wells of flat-bottomed polystyrene plates (Corning, New York, USA) coated with soluble antigen from sonicated *T. gondii* were added 200 µl of each serum sample (previously adsorbed onto Sepharose 4B-protein G) diluted 1/50 in 0.15 M phosphate-buffered saline, pH 7.2 (PBS) containing 0.1% Tween 20 (T) and 1% bovine serum albumin. After incubation for one and a half hours at room temperature (RT), the wells were washed three times with PBS-T and 200 µl of the optimal dilution of conjugate (goat anti-human IgA-peroxidase) in PBS-T were added to the wells. After incubation with the conjugate for one hour at RT and washing with PBS-T as previously described, 200 µl of the substrate solution containing 0.42 mM tetramethylbenzidine and 1.42 mM hydrogen peroxide, were added to the wells. Fifteen minutes after the addition of the substrate, 50 µl of 4 N H_2SO_4 were added to each well to stop the colour reaction. The absorbance of the wells was measured at 450 nm using a microtiter plate spectrophotometer. The final optical density of each well was determined by subtracting the mean optical density of three antigen controls run in parallel. Serum standards (5 to 1000 AU/ml) prepared from an IgA positive reference serum pool were included in each assay. All serum samples were tested in triplicate and the mean activity was converted into AU/ml using the appropriate standard curve. IgA-dELISA values > 15 AU/ml were considered positive. The IgA-cELISA was performed using commercial kits (ETI-TOXOK A) from Sorin Biomedica (Italy). Serum standards (10 to 160 AU/ml) were included in each assay. All serum samples were tested in duplicate and the resulting mean absorbance then converted into AU/ml using an appropriate standard curve. According to the manufacturer's instructions, most patients with acute toxoplasmosis have specific IgA levels > 40 AU/ml. Levels ranging from 10 to 40 AU/ml cannot be regarded as negative and their importance must be interpreted in association with specific IgM and IgG determinations; levels < 10 AU/ml are regarded as negative.

RESULTS

The serological data for the *T. gondii*-specific IgM and IgA antibodies in the 12 patients are shown in the table 1. All serum samples obtained during the first trimester after the onset of the clinical manifestations of toxoplasmic infection were positive for IgM when assayed by IIF, cELISA or CmL tests. In four cases, the IgM-IIF test remained positive up to the last serum sample taken five (patient 7), eleven (patient 11), twelve (patient 8) and sixteen (patient 12) months after the onset of clinical manifestations. A longer persistence of IgM was observed using cELISA and CmL. In seven cases (patients 2-6, 9, 10), the IgM-cELISA and IgM-CmL remained positive after the IgM-IIF test had given a negative result. In three cases (patients 5, 9 and 12), the IgM-cELISA and IgM-CmL were positive in serum samples obtained fifteen or more months after the beginning of clinical symptoms. The dELISA for IgA remained positive up to the last serum sample tested in 9 cases (patients 1-4, 6,7, 9-11). In two of these (patients 1 and 9), the IgA-dELISA was positive in serum samples taken fifteen or more months after the start of clinical manifestations. The IgA-cELISA also remained positive up to the last serum sample tested in 9 cases when 10 AU/ml was used as the cut-off value (patients 4-12). In three of these (patients 5, 9 and 12), cELISA values > 10 AU/ml were obtained in serum samples collected more than fifteen months after the onset of clinical symptoms. A shorter persistence of IgA was observed using the cELISA when values > 40 AU/ml represented significant antibody levels. At this cut-off level, a sustained persistence of IgA was observed in only one patient (n° 12) who had a cELISA result of 51 AU/ml in a serum sample taken twelve months after the beginning of clinical manifestations. In this patient, the three tests for IgM remained positive up to the last serum sample tested, sixteen months after onset of clinical symptoms. Two other cases with a sustained persistence of IgM (by IIF, cELISA and CmL) but with IgA antibody levels < 40 AU/ml by cELISA were observed (patients 8 and 11). IgA levels (cELISA) < 40 AU/ml were recorded in serum samples taken during the first trimester after the start of clinical manifestations (patients 2-4).

DISCUSSION

In the present study, the persistence of *Toxoplasma* specific IgM and IgA antibodies in serum samples obtained from 12 patients at various intervals after the onset of clinical symptoms of infection was investigated. Comparison of the IgM antibody test results showed that the IgM-IIF test exhibited the shortest persistence of positivity. This observation suggests that the IgM-IIF test correlates better with the recent stage of infection and that this test may be a more reliable indicator of recently acquired toxoplasmosis. However, in accordance with other studies[8, 21], the IgM-IIF test remained positive in some patients for a long period (≥ 11 months) after the onset of clinical symptoms, indicating that large individual variations in the antibody response to *T. gondii* may occur. Depending on the concentration of specific IgM antibodies, the IgM-IIF test may be negative in the initial stage of a *Toxoplasma* infection whereas more sensitive assays for IgM are positive[18]. From a technical point of view, the IgM-IIF test is highly dependent on good quality equipment and carefully trained personnel for proper reading and interpretation of the results. In addition, this IgM test is time-consuming because of the need to remove rheumatoid factor, antinuclear antibodies and IgG antibodies from the sera in order to improve the sensitivity and specificity of the test[1, 5, 6, 20].

TABLE 1

Toxoplasma specific IgM and IgA antibodies during the course of toxoplasmosis.

Patient	Time*	IgM			IgA	
		IIF	cELISA	CmL	dELISA	cELISA
1	1	4096	R	750	130	>160
	4	64	R	360	54	28
	9	32	R	331	28	2
	15	<32	NR	34	20	1
2	1	8192	R	>860	204	>160
	3	128	R	ND	118	24
	5	<32	R	ND	82	19
	6	<32	R	819	56	2
3	1	1024	R	518	640	>160
	2	256	R	ND	190	17
	9	<32	R	216	54	1
4	1	2048	R	399	158	>160
	3	256	R	ND	95	30
	8	<32	R	179	61	24
5	1	512	R	>860	40	>160
	2	128	R	ND	14	>160
	9	<32	R	580	8	27
	12	<32	R	530	10	29
	15	<32	R	ND	8	30
	21	<32	NR	481	6	28
6	1	1024	R	>860	118	>160
	2	256	R	ND	40	>160
	4	<32	R	450	44	39
	7	<32	R	320	36	34
	9	<32	NR	296	26	12
7	2	512	R	>860	43	>160
	4	64	R	ND	41	56
	5	32	R	791	26	29
8	3	512	R	>860	ND	64
	6	256	R	630	62	60
	8	128	R	582	53	39
	12	64	R	485	14	29
9	2	512	R	310	60	>160
	4	64	R	ND	ND	104
	6	<32	R	ND	30	58
	17	<32	R	180	21	19
10	2	256	R	400	48	>160
	5	64	R	ND	30	64
	6	32	R	ND	ND	53
	8	<32	R	210	18	42
11	3	256	R	ND	52	85
	8	64	R	221	54	52
	11	32	R	215	54	39
12	1	4096	R	829	20	>160
	5	512	R	550	ND	99
	12	128	R	338	19	51
	16	64	R	298	6	17

*Months from the onset of clinical manifestations to sampling of blood; Significant serological results: IgM-IIF ≥ 32; IgM-cELISA = R; IgM-CmL > 140 AU/ml; IgA-dELISA > 15 U/ml; IgA-cELISA > 40 AU/ml; ND = not determined; R = reactive; NR = non-reactive.

Commercial immunoenzymatic kits for the detection of *Toxoplasma*-specific IgM antibodies are being used increasingly in clinical situations. The assays are usually easy to perform, and according to the manufacturer's specifications all of them are very accurate. VERHOFSTEDE et al. (1989) have shown that *Toxoplasma* IgM kits are able to detect antibodies for short or long periods after a recent *T. gondii* infection, depending on the sensitivity of the kit. In the present study, both the IgM-cELISA and IgM-CmL were positive for a long time (17 and 21 months, respectively) after the onset of the clinical symptoms of toxoplasmosis. In 1980, NAOT and REMINGTON reported an ELISA for the detection of *Toxoplasma*-specific IgM antibodies that was more sensitive and more specific than the IgM-IIF test. This ELISA has been used as a reference test in many laboratories since the results obtained with this assay have shown an excellent agreement with other tests (Sabin Feldman dye test, IgA and IgE antibody tests, differential agglutination [AC/HS] test, and avidity test) used to distinguish recent from long-term *Toxoplasma* infections[16, 17]. Comparative studies between the reference IgM-ELISA and commercial kits have revealed significant discrepancies in the sensitivity and specificity of the assays[15, 16]. Other studies designed to assess the sensitivity and specificity of *Toxoplasma* IgM kits have also reported a considerably high number of false-negative and false-positive results in many of the commercial assays[2, 11, 24]. These discrepancies are probably related to factors such as the intrinsic properties of the techniques, the antigen preparation used in the assay, the selection of the sera and the method used to define the cut-off value. Some studies have reported that the use of *Toxoplasma* IgM kits with modified cut-off values may improve the predictive value for recently acquired toxoplasmosis[2, 9].

Our results show that the interpretation of positive IgM results as indicative of recently acquired toxoplasmosis in most cases requires additional laboratory confirmation either by other tests or by the demonstration of a significant rise in the antibody titers in sequential serum samples.

Previous reports have shown that *Toxoplasma*-specific IgA antibodies are frequently detected in the early phase of toxoplasmosis[7, 22]. In our case series, all 12 patients had positive dELISA and cELISA tests for IgA when the diagnosis of toxoplasmosis was confirmed in the laboratory. The analysis of sequential serum samples from these patients revealed that IgA antibodies may be detected by dELISA long after the onset of clinical symptoms and also by cELISA when a cut-off of 10 AU/ml is used for the latter assay. TURUNEN et al. (1983) also observed a sustained persistence of *Toxoplasma*-specific IgA antibodies by dELISA in some patients with a toxoplasmic infection. As defined by the manufacturer of the cELISA test used, most patients with acute toxoplasmosis have IgA antibody levels > 40 AU/ml. In a previous study, we screened serum samples from 51 patients with acute acquired toxoplasmosis for specific IgA antibodies[22]. Except for one sample obtained five months after the start of clinical symptoms (cELISA of 35 AU/ml), all of the other sera had antibody levels > 40 AU/ml (54 to > 160 AU/ml). The results obtained in the present series of patients support the view that cELISA IgA levels > 40 AU/ml can be useful markers of acute human toxoplasmosis. However, it should be stressed that IgA levels ≤ 40 AU/ml do not exclude a recent infection, since in some patients such levels can be reached very soon after infection with *T. gondii*.

The results obtained in the present study show that the serological diagnosis of acute toxoplasmosis may not be such an easy task. Our data suggest that use of the IgA-cELISA concomitantly with IgM antibody screening could permit, in some circumstances, a more efficient diagnosis of acute acquired toxoplasmosis.

RESUMO

Resposta de anticorpos IgM e IgA em 12 casos de toxoplasmose humana adquirida

A persistência, em alguns indivíduos, de anticorpos específicos IgM anti-*Toxoplasma gondii* por vários meses, após a fase aguda da infecção, tem complicado a interpretação dos resultados dos testes sorológicos para a toxoplasmose. Vários trabalhos têm enfatizado o valor da detecção de anticorpos específicos IgA anti-*T. gondii* para o diagnóstico da toxoplasmose aguda. No presente trabalho, são apresentados os resultados da pesquisa de anticorpos específicos das classes IgM e IgA anti-*T. gondii* em amostras de soros de 12 pacientes, obtidas em diferentes intervalos de tempo, após o início das manifestações clínicas da infecção. Os anticorpos IgM foram detectados pelo teste de imunofluorescência indireta (IFI), e pelas técnicas imunoenzimáticas da captura (ELISAc) e quimioluminescência (QmL). Os anticorpos IgA foram quantificados pelas técnicas de ELISA direta (ELISAd) e ELISAc. De acordo com as instruções do fabricante do "kit" de ELISAc usado para a pesquisa de IgA, a maioria dos pacientes com toxoplasmose aguda tem níveis de anticorpos > 40 unidades arbitrárias por ml (UA/ml). Utilizando este parâmetro, a técnica de ELISAc para IgA detectou níveis significativos de anticorpos (> 40 UA/ml) por um período mais curto do que as outras técnicas usadas para a detecção de IgM e IgA. Entretanto, níveis de IgA ≤ 40 UA/ml não excluem a possibilidade de toxoplasmose aguda, pois tais níveis podem ser alcançados em um curto período de tempo após a infecção com *T. gondii*. Nossos dados sugerem que, a pesquisa de anticorpos IgA por ELISAc concomitante com a pesquisa de anticorpos IgM permitiria, em algumas circunstâncias, um diagnóstico mais preciso da toxoplasmose aguda adquirida.

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