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USEFULNESS OF THE DETECTION OF *TOXOPLASMA GONDII* ANTIGENS IN AIDS PATIENTS

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SUMMARY

Toxoplasmic encephalitis (TE) is a mayor cause of central nervous system infection in patients with acquired immunodeficiency syndrome (AIDS). Toxoplasma antibodies were detected in 56 of 79 patients with AIDS (71%), in the present study. Fourteen out of 57 seropositive patients developed TF (25%) and had *Toxoplasma gondii* antigen detected in their urine. For this, most of them received an effective therapy, with the subsequent disappearance of the symptoms and discontinuity of excretion of the *T. gondii* antigens.

Our results suggest that the monitoring of *T. gondii* antigen in the urine of AIDS patients may be useful to decide on the proper time for therapy, as well as to avoid the beginning of neurologic signs in these patients.

KEYWORDS: *T. gondii*; toxoplasmic encephalitis; AIDS.

INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS) is a disease which favours different opportunist infections, among them those of the neurologic system [11]. Approximately 30% of the patients with AIDS in the United States and Europe suffer of toxoplasmosis of the central nervous system (CNS) [13, 15, 16]. This can be the result of the reactivation of an infection or of the onset of a primary one [16].

Unfortunately, serodiagnosis of toxoplasmosis in patients with AIDS is difficult, due to their deficient immune reaction, which is characterized by the frequent absence of specific anti-*Toxoplasma gondii* IgM antibodies [10].

We have also found that the titres of specific anti-*T. gondii* IgG antibodies are usually low and consequently their detection is not conclusive for the diagnosis [10]. Despite that, it is possible to demonstrate the intrathecal production of specific anti-*T. gondii* antibodies in patients with toxoplasmic encephalitis [17].

The detection of circulating antigens (Ag) in biological fluids enables us to determine if the parasite is in an evolutive stage [18]. For this purpose a solid phase enzyme linked immunosorbent assay (ELISA) for serum samples [9], and the coagglutination test for urine samples [6] have been used.

Our main purpose in the present study is to evaluate the relationship among the levels of anti-*T.gondii* antibodies, the occurrence of toxoplasmic encephalitis and the presence of circulating antigens, in order to identify the proper time for the beginning of treatment.

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MATERIAL AND METHODS

Samples

Serum and urine samples were collected from 79 patients with AIDS (according to CDC, Atlanta) [1], to test for the presence of anti-*T. gondii* antibodies (abs) and antigens (ags) of this protozoa, respectively.

All patients had both samples collected at the moment they were included in group IV. Their urine samples continued to be collected monthly for two years, and, from those patients that developed associate toxoplasmic neurologic symptoms, daily afterwards, when a second serum sample was also collected. When the symptoms were not observed and *T. gondii* antigens were no longer detected in urine due to therapy, urine samples were collected monthly again.

Clinical Diagnosis

The patients infected with the human immunodeficiency virus (HIV) were suspected of having toxoplasmic encephalitis when they presented neurologic focal manifestations, associated or not with the loss of conscience, as well as suggestive lesions of cerebral abcess in the computerized axial tomography (CAT).

Indirect Immunofluorescence Assay (IFA)

The immunofluorescence test for detection of anti-*T. gondii* IgG abs was performed according to the methodology used in the Protozoology Laboratory of the Institute of Tropical Medicine "Pedro Kouri" [14]. A commercial kit (Behring) was used for the detection of anti-*T. gondii* IgM abs.

Production of the Antigenic Extract

A soluble antigenic extract was prepared from the RH strain of *T. gondii*. The trophozoites were obtained from mice peritoneal exudate, three days after infection. The exudate was centrifuged at 650g, for 10 min., at 4 C, in 2 ml of 0.1M phosphate buffer saline (PBS), pH 7.2, to eliminate the host cells residues. The supernatant was discarded. The pellet was resuspended in 2 ml of PBS and repeatedly passed through a 26 gauge needle in order to rupture the macrophages. It was then centrifuged at 160g for 10 min., at 4C, and the supernatant was centrifuged at 650g for 10 min., at 4C. The pellet was washed three times with 50 ml of PBS and resuspended in the same solution. The suspension was then sonicated at 4C to kill the parasite and centrifuged at 650g for 2 hours, at 4C. The protein concentration of the supernatant was determined by the method of LOWRY et al. [12], and the *T. gondii* antigen extract was stored at-20°C in 1 ml aliquots.

Production of the Anti-T. gondii antiserum

Half an ml of the *T. gondii* soluble extract (4 mg/ml of protein) was diluted with the same volume of PBS. This solution was mixed with 1 ml of Freund's complete adjuvant for subcutaneous inoculation of each rabbit (approximately 3kg body weight).

Four inoculations were performed at weekly intervals. The animals were bled by cardiac puncture seven days after the last inoculation and the sera were stored at -20°C. The anti-toxoplasma antibody titre of the hyperimmune sera was determined by counterimmunoelectrophoresis (1/64) and ELISA (1/51200)

Coagglutination test (CoA-Toxo)

The detection of *T. gondii* antigens was performed by the coaglutination technique described by FACHADO et al [5].

Briefly, 1 ml of a 10% suspension of *Staphylococcus aureus* (*S. aureus*, strain Cown I), rich in protein A, was mixed 0.5 ml of the anti-toxoplasma rabbit hyperimmune serum and the suspension was kept at 37°C for three hours.

Subsequently to sensitization with antibodies, the bacteria were washed three times in which PBS, pH 7.2. The antibody-coated staphylococci were then resuspended in an appropriate volume of PBS, containing 0.4% methylene blue and 0.1% sodium azide, to a 5% final suspension (w/v). The same procedure was followed to prepare the control reagent, with the only difference that a normal rabbit serum was used instead of the rabbit immune serum.

For the test, one drop of each urine sample was applied in duplicate to a white card (Fig. 1). One drop of the sensitized *S. aureus* suspension was added to one of the samples' duplicate (upper circles), and the same volume of the nonsensitized *S. aureus* control suspension was added to the other one (lower circles).

RESULTS

In the study of 79 patients infected with HIV at the moment they were included in group IV, *T. gondii* specific IgG was detected in the serum of 56 of them

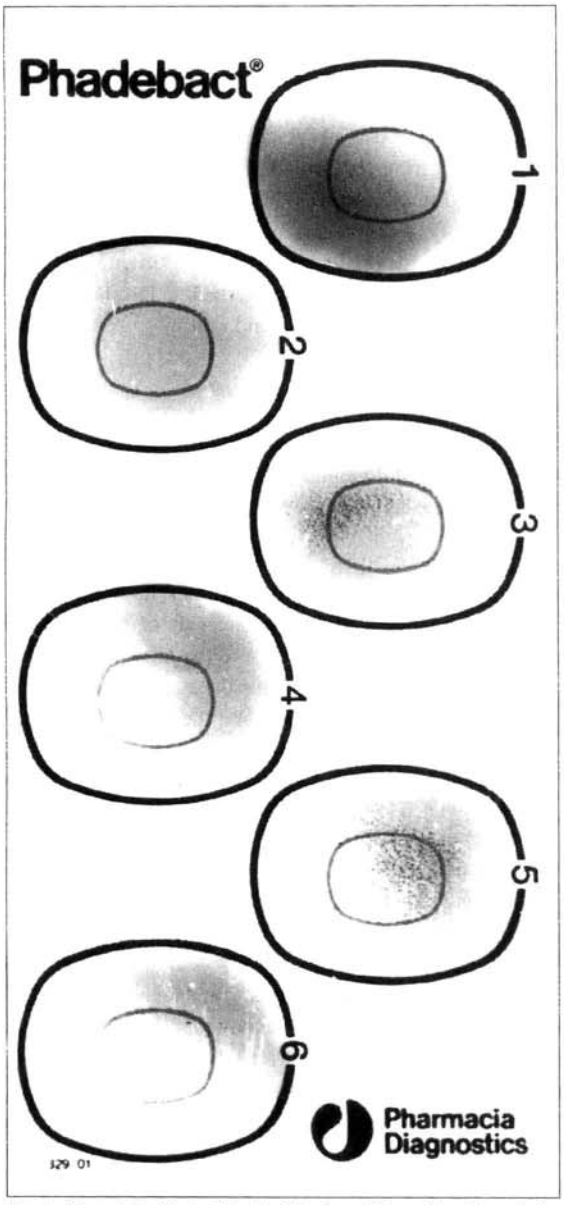

Fig. 1 - Coagglutination test for the detection of *T. gondii* antigens in the urine of patients with AIDS. Positive agglutination (circles 1,3 and 5). No agglutination (circles 2,4 and 6).

(71%). In none of these serum samples was detected *T. gondii* specific IgM. All urine samples collected at that time were negative for the CoA-Toxo test (Table 1). Later on, 14 out of the 56 seropositive patients presented a clinical picture compatible with toxoplasmic encephalitis and in all of them the *T. gondii* antigen was detected in urine. No significant increase in the IgG levels nor the appearance of IgM antibodies specific for *T. gondii* were observed in these patients sera (Table 2).

TABLE 1

Distribution of the number of patients infected by the HIV and recently included in group IV, according to their serum positivity to *T. gondii*.

Number of patients	Serology - IFA IgG	IgM	CoA-Toxo
79	56 (71%)	Neg.	Neg.

neg: negative; HIV: Human Immunodeficiency Virus; IFA: Immunofluorence assay; CoA-Toxo: Coagglutination to detect *T. gondii* antigens

TABLE 2

Levels of antibodies of 14 patients with AIDS before and after presenting clinical characteristics compatible with neurotoxoplasmosis.

Patients with positive CoA-Toxo	Serology Before IFA IgG	IgM	After IFA IgG	IgM
1	1/32	neg	1/32	neg
2	1/16	neg	1/16	neg
3	1/16	neg	1/16	neg
4	1/16	neg	1/16	neg
5	1/16	neg	1/16	neg
6	1/16	neg	1/16	neg
7	1/64	neg	1/64	neg
8	1/16	neg	1/16	neg
9	1/32	neg	1/32	neg
10	1/64	neg	1/64	neg
11	1/16	neg	1/16	neg
12	1/16	neg	1/16	neg
13	1/64	neg	1/32	neg
14	1/32	neg	1/32	neg

IFA: Indirect Immunofluorence Assay 1/16 is considered positive.

One can observe in Table 3 that there is a total correspondence between the clinical diagnosis and the coagulation results.

The AIDS patients which were suspected of having neurotoxoplasmosis and in whose urine *T. gondii* antigen was detected, were submitted to treatment with pirimetamine and sulfadiazine for six weeks, and subsequently to a prophylactic treatment with primetamine for two months. The patients that presented a favourable clinical evolution in this period, and in which the CoA-Toxo test for urine became negative, had their treatment interrupted.

It can be observed in Table 4 that, the toxoplasma antigen in urine became negative at different periods of time after the beginning of treatment, for the different cases, and varied from 5 days (20% of the cases) to

TABLE 3

Correlation of the clinical observations with the results of the Coagglutination (CoA-Toxo) test

		CoA-Toxo +	CoA-Toxo -
Clinical Neurotoxoplasmosis	+	14	0
	-	0	65

CoA-Toxo: Coagglutination to detect *T. gondii* antigens in urine.

approximately 1 month, when all urine samples were negative for the antigen.

DISCUSSION

Toxoplasmic encephalitis in AIDS patients is usually caused by the reactivation of a latent infection, and coincides, in general with the presence in serum of *T. gondii*-specific antibodies, in low titers [2, 9].

We had serologic evidence for *T. gondii* infection in 71% of the patients, a figure similar to that obtained by DEROUIN et al. [4]. Twenty five percent of the patients that had specific serum antibodies to toxoplasma developed toxoplasmic encephalitis. This result is in agreement with the results of GRENT et al. [8] and DECLERCQ et al. [3] (23.8% and 24% respectively).

The lack of significant differences in the titres of the serum anti-*T. gondii* IgG antibodies in 14 patients, before and after they presented a clinical picture which suggests toxoplasmic encephalitis, shows that serology is not an efficient diagnostic tool in this disease. DARCY et al. had already pointed out this fact [2].

The presence of antibodies in serum is useful for the identification of the patients with a latent infection. High titres of specific *T. gondii* antibodies are hardly found in AIDS patients suffering from toxoplasic encephalitis, though. For this reason the test for the presence of the toxoplasmic antigens in biologic fluids is a necessary one.

All of our cases in which *T. gondii* antigens were detected in urine, presented focal signs, such as left and/or right hemiparesy, convulsions, muscular hypertonicity, hemiplegia, and some general signs like (mental?) confusion lethargy, lack of orientation, etc, that were confirmed by computerized axial tomography in all of them (round, single and multiple, isodense and hypodense lesions).

These results lead us to suggest that the detection of these antigens in urine may be an important tool for the diagnosis of toxoplasmosis and, consequently, for the use of the appropriate therapy.

When the adequate therapy was used, not only the symptomatology but also the antigens in urine disappeared, the latter at different intervals of time for the different patients. This was due to the characteristics of the different patients regarding the degree of commitment of their immune system, and the number and kind of the opportunistic infection that affected them.

The data reported by some researchers, as well as those obtained by us, stress the fact that many AIDS patients, positive for serum antibodies to *T. gondii*, did not present any symptomatology of toxoplasmic encephalitis for a long time. This suggests that the prolonged use of chemoprophylaxis [4, 8] should be avoided thus preventing the unnecessary and excessive use of drugs, which would affect the patients' health, and also lowers the cost of treatment [7, 19]. So we consider that a complementary test is necessary, to enable us to foresee the development of a neurologic complication related to toxoplasmosis and to begin the corresponding prophylaxis. We think that the diagnostic method described in the present paper may be useful for this purpose and should be further studied.

TABLE 4

Disappearance of *T. gondii* antigens in urine of patients with AIDS after treatment.

Number of patients with positive CoA-Toxo	Effective prophylactic treatment							
	5 days CoA-Toxo		10 days CoA-Toxo		15 days CoA-Toxo		1 month CoA-Toxo	
	+	-	+	-	+	-	+	-
14	10	4	7	7	3	11	0	14

CoA-Toxo: Coagglutination to detect *T. gondii* antigens.

RESUMEN

Utilidad de la detección de antígenos de *Toxoplasma gondii* en pacientes con SIDA

La Encefalitis Toxoplásmica (ET) es la más importante complicación infecciosa del Sistema Nervioso Central en pacientes de SIDA. Anticuerpos anti-*Toxoplasma gondii* fueron detectados en 57 de 79 pacientes de SIDA (71%). De estos seropositivos, desarrollaron la enfermedad (ET) 14 (25%),en los que coincidentemente se detectó la presencia de antígeno del parásito en orina y por tanto fueron objeto de una terapia efectiva, con la subsecuente desaparición de los sintomas y de los antígenos excretados. Por los resultados del presente trabajo, consideramos lo útil de monitorear en estos pacientes la presencia de antígenos de *T. gondii* con el objetivo de aplicar oportunamente métodos quimoprofilácticos que eviten el surgimiento de manifestaciones neurológicas en estos pacientes.

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