

## Lacrimal Secretory IgA in Active Posterior Uveitis Induced by *Toxoplasma gondii*

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*It is quite difficult to diagnose active toxoplasmosis in patients with ocular toxoplasmosis. Active posterior uveitis presumably due to Toxoplasma gondii infection (APUPT) is seldom produced during a prime-infection; hence most patients do not show high IgM antibodies. High levels of IgA have been described in active toxoplasmosis. The purpose of this study was to investigate possible association between APUPT and the specific anti-parasite sIgA in tears. The study was carried out as case-control. Tears of 25 clinically confirmed APUPT patients and 50 healthy control subjects were analyzed. All were IgG seropositive. Specific sIgA was determined by ELISA assay using T. gondii RH strain crude extract. Anti-T. gondii sIgA was found in 84% of the cases and in 22% of the control subjects. The intensity of the reaction was higher in APUPT cases (P = 0.007). There was strong association between APUPT patients and lacrimal sIgA (odds-ratio 18.61, P = 0.0001). ELISA test sensitivity was 84% and specificity 78%. Our data suggest that anti-T.gondii secretory IgA found in tears may become an important marker for active ocular toxoplasmosis.*

Key words: anti-*Toxoplasma gondii* secretory IgA - ocular toxoplasmosis diagnosis - antibodies anti-*T. gondii* - secretory IgA and ocular toxoplasmosis

Toxoplasmosis, one of the most widespread zoonosis in the world (Tonelli 2000), is caused by *Toxoplasma gondii*, an intracellular protozoan of worldwide distribution.

In Brazil 50 to 80% of the adult population are seropositive (Oréface & Bonfioli 2000). Some regional variation in prevalence can be observed. For instance, in Rio Grande do Sul prevalence rate is 82%, in São Paulo 42%, and in Paraná 66% (Garcia et al. 1999). In Pernambuco 79.1% of children between 9 and 15 years of age, from a micro region of Metropolitan Recife, were seropositive (Kobayashi et al. 2002).

Toxoplasmosis is an important cause of uveitis in different parts of the world. In Brazil it is responsible for 50 to 80% of total uveitis (Belfort et al. 1978, Silveira et al. 1991, Jain et al. 1998, Gehlen et al. 1999). Lesions may be active or quiescent and scarred. Active ocular lesions consist of well-defined foci of granulomatous retino-chorioiditis with necrosis, unique or multifocal, and may be associated with other preexistent lesions. Lesions heal by scarring. The scars show well-limited borders with variable degrees of pigmentary epithelium hypertrophy, retinochoroideal atrophy with occasional choroidal and scleral blood vessels in the center. Ocular toxoplasmosis disease, congenitally and post-natally acquired, is generally believed to occur during the chronic phase of infec-

tion, although there are reports of lesions developed shortly after the first infection.

Serological tests are the main tool for laboratory diagnosis of toxoplasmosis. In recently acquired *Toxoplasma* infection, IgG rises one week after, with low avidity antibodies. Later, antibody titers decrease and high avidity IgG may persist for years. Although considered as a good marker for active toxoplasmosis, low avidity IgG does not always identify recent infection, as observed by Paul (1999). Increased serum IgA, IgM, and IgE has been found in recent *Toxoplasma* infection. In ocular toxoplasmosis IgG usually occurs in low concentration while IgM is absent (Oréface & Bonfioli 2000). Classical serology is ineffective, but investigation of antibodies in the aqueous fluid is adequate.

Katina et al. (1992), Ronday et al. (1999), and Garweg et al. (2000), showed that IgA antibodies in the aqueous fluid may help in the diagnosis of ocular toxoplasmosis. IgA, IgG, IgD, and IgE could be detected in tears. On the other hand, Meek et al. (2000) studied and found IgA specific to *T. gondii* in healthy laboratory volunteers from the Netherlands, a country where toxoplasmosis is endemic.

Secretory IgA (sIgA) together with active phagocytes and lysosomes in the lacrimal film constitute the first defensive barrier of the eye to infectious agents. It seems thus worthy to investigate the presence of secretory IgA specific to *T. gondii* in the tears of patients with ocular toxoplasmosis and any association that may exist with the illness.

### PATIENTS AND METHODS

*Active posterior uveitis induced by T. gondii patients*  
- They were older than 10, regardless of gender or neigh-

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borhood, with APUPT diagnosed through indirect binocular ophthalmologic examination at the Eye Clinic of the Hospital das Clínicas, Federal University of Pernambuco, Brazil. In order to consider APUPT as present, the following clinical aspects were defined: scarred chorioretinitis with well defined borders, suggestive signals of hyperthrophy and atrophy of the pigmentary epithelium together with satellite lesions showing inflammatory activity, different degrees of retinal vasculitis, and vitreal damage associated or not with lesions of the anterior pole structures (Oréface et al. 2000). Twenty-five IgG anti-*Toxoplasma* positive patients fulfilled these conditions.

**Control subject** - They were chosen among healthy volunteers, without any ocular illness, IgG reactive to *T. gondii* and older than 10. Preference was given to patients' relatives or neighbors, since they were from the same habitat; 50 individuals were selected.

**Tears samples** - They were collected with a standardized filter paper Toyo nr 4 (5 cm length × 0.5 wide). The paper strip, folded at 0.5 cm from the end, was inserted under the external third of the lower eyelid during 5 min. There was no stimulation of tear production. Before analysis, the filter paper strips, containing tears, were cut to the weigh of 0.06 mg.

Blood samples were collected for sera IgA and IgG determination. All samples were frozen at -23°C until examination.

***T. gondii* crude extract preparation** - RH strain tachyzoites from mice peritoneal cavity, were collected on the 4th day of infection with PBS 0.01 M pH 7.2, pelleted at 800 g and washed twice. The protocol of extraction followed the antigen preparation described by Meek et al. (2000).

**sIgA determination** - Tears were extracted from weighed filter paper with 200 ml of PBS 0.01 M pH 7.2 under heavy shaking with touch mixer. sIgA was determined by antibody capture ELISA. *T. gondii* crude antigen, diluted in carbonate buffer 0.05 M pH 9.0 to 5.1 µg/ml, were immobilized in microtiterwells (MaxiSorp Nunc) plates during 3 h, followed by a blocking step with skimmed milk during 3 h. After washing with PBS-tween (0.05%) 0.06 M, pH 7.2, 50 µl of extracted tears were incubated during 1 h at 37°C. Fifty µl of rabbit anti-secretory piece (Sigma) 1/700, followed by 50 ml of properly diluted horseradish peroxidase-conjugated goat anti-rabbit IgG (Sigma) incubated at 37°C for 1 h each, were used for detecting the presence of specific sIgA. H<sub>2</sub>O<sub>2</sub> 0.05% and OPD (32 mg into 10 ml citrate buffer) were used as substrate. Opti-

cal density of the reaction was read at 492 nm.

**Sera IgG and IgA determinations** - These were done after the basic ELISA protocol described above. The plates were coated with 8 mg/ml of antigen. Goat anti-human IgG or anti-human IgA conjugated to horseradish peroxidase (Sigma) diluted 1/2000 and 1/1500 were used respectively.

Each assay included three positive and three negative sera or tears for internal quality control.

On analyzing results with program Epi Info version 6.04 1997, APUPT was treated as the dependent variable.

**Anti-*T. gondii* serum IgM immunofluorescence** - Sera IgM anti-tachyzoites were tested using the technique of indirect immunofluorescence according to the well-established protocol of Camargo (1964).

The Ethics Committee of the Universidade Federal de Pernambuco, Brazil, approved all procedures.

## RESULTS

Seventy-five individuals, 25 APUPT cases and 50 control subjects, were studied from January to December 2002. The average age of 20 (80%) of the patients, and 22 (44%) of the control subjects was between 10 and 30, while 5 (20%) of cases were older than 30. Association studies between APUPT and age distribution pointed to a 1.94 times higher risk to develop the illness in patients aged 21 to 30 years.

The serological determination of IgG specific to tachyzoite crude soluble extract showed more intense reactions in APUPT cases than in control group, although there was no statistical difference ( $P = 0.082$ ) between them. The absorbance arithmetic media of ELISA for sera IgA was more intense in the APUPT group than in the control group ( $P = 0.04$ ). Tears sIgA absorbance intensity was higher in APUPT cases than in control subjects ( $P = 0.007$ ). Results are shown in Table I.

Association studies between secretory IgA in APUPT patients and healthy control subjects showed an odds-ratio = 18,61 (CI: 4.59-82.8; 95% confidence limits) as seen in Table II.

Sensitivity and specificity of ELISA test for anti-*T. gondii* sIgA antibodies were of 84% and 78%, respectively.

IgM specific antibodies were not detected in APUPT patients.

All 25 patients had positive reactions for anti-*T. gondii* serum IgA, but 21 of them (84%) were tear sIgA positive, while 4 patients (16%) did not show *T. gondii* specific sIgA. In the control group, 11 out of 50 (22%) had sIgA in

TABLE I

Absorbance average of sera IgG, IgA, and tears secretory IgA anti-*Toxoplasma gondii* from active posterior uveitis presumably due to toxoplasmosis (APUPT) patients and control subjects treated at Hospital das Clínicas, Pernambuco Federal University, 2002

Anti- <i>T. gondii</i> antibodies	APUPT cases		Control subjects		
	(n = 25)	SD	(n = 50)	SD	p
Sera IgG	1.38	0.373	1.119	0.435	0.082
Sera IgA	0.663	0.175	0.574	0.217	0.040
Tears secretoryIgA	0.370	0.390	0.188	0.244	0.007

TABLE II

Odds-ratio confidence interval to the association between APUPT and secretory IgA anti-*Toxoplasma gondii* in tears from patients treated at the Hospital das Clínicas, Pernambuco Federal University, 2002

Anti- <i>T. gondii</i> sIgA	Cases		Control subjects		OD	IC of 95%
	n	%	n	%		
Positive	21	84	11	22	18.61	4.59-82.8
Negative	4	16	39	78	1 <sup>a</sup>	

$\chi^2 = 26.19$        $p = 0.0001$        $n = 75$

APUPT: active posterior uveitis presumably due to toxoplasmosis; *a*: reference value; OD: odds-ratio

tears and 6 (12%) had IgA in their sera. Out of 39 control subjects with no tear sIgA, 20 did not show *T. gondii* IgA antibodies in their sera, while 19 individuals did.

The agreement index "Kappa" was determined by comparing serum IgA with lacrimal anti-*T. gondii* sIgA considering the presence (positive) or absence (negative) of immunologic reaction in 75 individuals. The "Kappa" index obtained, 0.238, was not significant (0.2-0.40 > not relevant).

#### DISCUSSION

Diagnosis of ocular toxoplasmosis is often based on typical clinical appearance. The presence of an active white-yellowish lesion with pigmented scar and perivasculitis in the retinochoroid helps the diagnosis. However, other causes may lead the same clinical aspect. Serologic exams provide valuable information, but more frequently the etiologic diagnosis of the disease cannot be ascertained.

Funduscopy aspects still remain those, which correlate better with the histopathology of the disease. The coined expression "presumable toxoplasmosis" shows the uncertainty between the binomial morphology of the focal lesion and the anti-*T. gondii* serology.

Detection of anti-*T. gondii* IgG in the blood has also been valued for diagnosing. However, the infection is widespread and most adults in the world, particularly in Brazil, who are IgG positive, show no visual impairment. Although the population in our study had had previous contact with the parasite (IgG positives), only patients with active APUPT showed higher absorbance intensities in IgG ELISA test, albeit not statistically significant when compared to the control subjects.

Serum IgM antibodies are elevated in recently acquired *T. gondii* infection, but they can remain elevated even up to one year after. In case of reactivation, no IgM was reported by Ongkosuwito et al. (1999), but Gomes-Marin et al. (2000) in Colombia suggest the possibility of finding IgM in recurrent cases. In our study, no patient had detectable IgM anti-*T. gondii* antibodies, even when sera were analyzed by immunofluorescence and ELISA test. The hypothesis that all the studied APUPT patients suffered the parasite infection for a long time, mostly when all of them had scarred lesions in the posterior pole of the eye characterizing recurrent infection, could be accepted.

Serum IgA has been considered a useful marker for active toxoplasmosis. Ronday et al. (1999) reported 16% of positive IgA cases in confirmed ocular toxoplasmosis patients but not in any individual of the control group without the disease.

Every patient we studied was IgA positive (100%), Le Fichoux et al. (1987), Pujol et al. (1989), Stepich-Biek et al. (1990), Katina et al. (1992) however, describe absence of these antibodies in chronic toxoplasmosis. On the other hand, Gomes-Marin et al. (2000) reported that during the reactivation of retinochoroiditis specific IgM/IgA or IgM/IgE were present. In our study, anti-*T. gondii* IgE was not analyzed.

A defined relationship between anti-*T. gondii* sera IgA and lacrimal sIgA was not observed. IgA was 100% positive in the patient group and in 44% of control subjects. Since prevalence in the state of Pernambuco is high and the whole population analyzed was IgG positive, sera IgA levels in normal populations must be carefully studied.

Since the sIgA tear concentration is high and sample collection is not harmful for the patient, the use of standardized filter paper for that purpose seemed convenient along the study.

The specificity of the immunoassay was 74% and the sensitivity 84%. Considering that it is being used tachyzoite crude lysate as antigen, these results seem adequate, mostly when it has been reported that tear sIgA from normal individuals could react with protein bands of Mr 88 to 34 kDa from the tachyzoite crude lysate (Meek et al. 2000).

Specific sIgA in tears was detected in 24 (84%) of APUPT patients and in 24 (22%) of control subjects. Analyzing sIgA anti-*T. gondii* in the control and patient group, there was a neat statistically significant difference in the intensity of reaction. There was also a strong association between APUPT and sIgA in tears (odds ratio 18.0,  $P = 0.0001$ ).

In conclusion, considering the strong association found between the disease and the lacrimal sIgA production as well as the mild conditions for obtaining tear samples, the proposed method for determining anti-*T. gondii* sIgA should be helpful in the APUPT diagnosis.

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