

# Detection of *Toxoplasma gondii* DNA in peripheral blood and aqueous humor of patients with Toxoplasmic active focal necrotizing retinochoroiditis using real-time PCR

## Detecção de DNA do *Toxoplasma gondii* no sangue periférico e humor aquoso por PCR em tempo real

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### ABSTRACT

**Purpose:** To evaluate the ability of real-time quantitative PCR (qPCR) for detecting *Toxoplasma gondii* DNA in the peripheral blood and aqueous humor of patients with toxoplasmic active focal necrotizing retinochoroiditis.

**Methods:** Fifty-five patients with infectious uveitis seen from 2009 to 2013 at the Department of Ophthalmology and Visual Sciences of the Federal University of São Paulo were enrolled in this study. Forty-three patients had toxoplasmic active focal necrotizing retinochoroiditis, and the remaining 12 had non-toxoplasmic infectious uveitis and served as controls. qPCR analysis for *T. gondii* DNA was performed on the patients' peripheral blood and aqueous humor samples.

**Results:** The qPCR was positive for *T. gondii* DNA in 37.21% (16/43) of the aqueous humor samples and 2.33% (1/43) of the peripheral blood samples; further, 16.27% (7/43) of the patients had positive results in both their blood and aqueous humor samples.

**Conclusion:** qPCR was able to detect *T. gondii* DNA in patients with toxoplasmic active focal necrotizing retinochoroiditis in the blood as well as the aqueous humor and can help with the diagnosis of the disease.

**Keywords:** Toxoplasmosis, ocular/diagnosis; *Toxoplasma*; Blood/parasitology; Choroiditis; Real-time polymerase techniques/methods; Aqueous humor

### RESUMO

**Objetivo:** Analisar o uso do PCR em tempo real (qPCR) na detecção do DNA do *T. gondii* no sangue periférico e no humor aquoso de pacientes com lesões de retinochoroidite focal, ativa por toxoplasmose.

**Métodos:** Cinquenta e cinco pacientes com uveíte infecciosa foram incluídos neste estudo. Os pacientes foram atendidos entre 2009 a 2013, no Departamento de Oftalmologia e Ciências Visuais da Universidade Federal de São Paulo. Quarenta e três pacientes tiveram o diagnóstico de lesões de retinochoroidite focal, ativa por toxoplasmose e, os outros 12 tiveram o diagnóstico de uveíte infecciosa não toxoplásmica e, por isso foram usados como grupo controle. A técnica de qPCR foi utilizada na detecção de DNA do *T. gondii* em amostras de sangue periférico e humor aquoso.

**Resultados:** O qPCR foi positivo para o DNA do *T. gondii* em 37,21% (16/43) das amostras de humor aquoso, 2,33% (1/43) nas amostras de sangue periférico e, 16,27% (7/43) em ambas amostras simultaneamente.

**Conclusão:** O qPCR foi capaz de detectar o DNA do *T. gondii* em pacientes com lesões de retinochoroidite focal, ativa por Toxoplasmose, no sangue bem como, no humor aquoso, podendo ajudar no diagnóstico.

**Descritores:** Toxoplasmose ocular/diagnóstico; *Toxoplasma*; Sangue/parasitologia; Coroidite; Reação em cadeia da polimerase em tempo real; Humor aquoso

### INTRODUCTION

*Toxoplasma gondii* infection is the most common cause of posterior uveitis worldwide and is an important cause of ocular disease in both immunocompromised and immunocompetent individuals<sup>(1)</sup>, accounting for 30% to 50% of uveitis in different countries<sup>(2)</sup>.

The prevalence of *T. gondii* infection in adults in Brazil ranges from 50% to 80% depending on the region studied<sup>(1,3-5)</sup>. In Erechim, a city located in the south of Brazil, 88% of the population is seropositive for *T. gondii*, with 18% developing ocular toxoplasmosis<sup>(5,6)</sup>.

Clinical diagnosis of ocular toxoplasmosis is usually made by ophthalmic examination. Molecular biology techniques can be successfully used to detect *T. gondii* DNA<sup>(7)</sup> when the clinical diagnosis is unclear. Polymerase chain reaction (PCR) can detect microorganism DNA and is a rapid method with high sensitivity and specificity that has been used to detect *T. gondii* DNA in different biological samples<sup>(8,9)</sup>. With the recent advances in molecular biology, the use of real-time PCR makes quantitative measurement of *T. gondii* DNA possible in patients with ocular toxoplasmosis<sup>(10-12)</sup>. Rapid recogni-

tion of specific infections is important for adequate management of uveitis<sup>(13)</sup>.

The aim of this study was to evaluate the ability of real-time quantitative PCR (qPCR) for the detection of *T. gondii* DNA in peripheral blood and aqueous humor samples of patients with toxoplasmic active focal necrotizing retinochoroiditis.

### METHODS

#### PATIENTS

Fifty-five patients with uveitis seen from 2009 to 2013 at the Department of Ophthalmology and Visual Sciences of the Federal University of São Paulo were included in this study. From the 55 patients, 43 were diagnosed with toxoplasmic active focal necrotizing retinochoroiditis. The remaining 12 were diagnosed with non-toxoplasmic infectious uveitis (toxocariasis=6, tuberculosis=3, and herpes virus=3), and served as controls.

Submitted for publication: May 4, 2015  
Accepted for publication: October 1, 2015

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**Funding:** This work was supported by grants from FAPESP, CNPq-473179/2011-3, instituto da visão and CAPES.

**Disclosure of potential conflicts of interest:** None of the authors have any potential conflict of interest to disclose.

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The study was approved by the local ethics committee investigational review board (0094/09), and informed consent was obtained from all patients.

## SAMPLES

The samples were collected by an ophthalmologist. Peripheral blood (5 ml) was collected in EDTA tubes. Approximately 0.1 ml of the aqueous humor was collected by anterior chamber paracentesis with a 30-gauge needle. Aqueous samples were obtained from patients who had at least two anterior chamber cells.

A total of 43 peripheral blood and 43 aqueous humor samples were collected from 43 patients with toxoplasmic necrotizing active focal retinochoroiditis and two or more inflammatory cells in the aqueous humor. Twelve non-toxoplasmic patients with active uveitis and two or more inflammatory cells in the aqueous humor (12 peripheral blood and 12 aqueous humor samples) were used as controls.

## DNA EXTRACTION

Total DNA was extracted from the peripheral blood and aqueous humor with a commercially available DNA mini column kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The DNA samples were stored at -20°C until use.

## REAL-TIME qPCR

Real-time qPCR was performed using TaqMan 10 µl, with sense and antisense primers at a concentration of 10 mM, 0.4 µl of probe, 6.8 µl of nuclease- and DEPC-free water (Invitrogen, Carlsbad, CA, USA), and 2 µl of DNA, with a total reaction volume of 20 µl. qPCR was performed on an ABI Prism 7500 DNA sequence detection system (Applied Biosystems, Waltham, MA, USA) and targeted the *T. gondii* 529-bp repetitive genomic sequence (rep529)<sup>(14,15)</sup> and the B1 gene<sup>(16)</sup>. β-globin qPCR was performed in parallel for each sample as described previously<sup>(17)</sup> in order to confirm the integrity of the DNA and to verify the PCR inhibitors.

## RESULTS

Real-time qPCR analysis detected *T. gondii* DNA in 37.21% (16/43) of the aqueous humor samples and 2.33% (1/43) of the peripheral blood samples; further, 16.27% (7/43) of the patients had positive results in both the peripheral blood and aqueous humor samples (Table 1).

Further, among the 43 patients, the qPCR analysis showed negative results in both the peripheral blood and aqueous humor samples in 19 patients (data not shown).

In the control group (n=12), all samples analyzed showed negative qPCR results for *T. gondii* (data not shown).

## DISCUSSION

Blindness and visual impairment caused by infectious uveitis can be prevented at least partially by early identification of the pathogen and the subsequent administration of appropriate treatment<sup>(18)</sup>. The

**Table 1. Real-time quantitative polymerase chain reaction (qPCR) positivity of *Toxoplasma gondii* DNA in the peripheral blood and aqueous humor samples of patients with toxoplasmic active focal necrotizing retinochoroiditis.**

	Real-time PCR		
	Peripheral blood	Aqueous humor	Both peripheral blood and aqueous humor
Nº of positive/total	1/43	16/43	7/43
%	2.33	37.21	16.27

use of real-time PCR as a diagnostic tool for infectious uveitis has been demonstrated by many groups<sup>(11,19-21)</sup>.

In this study, qPCR identified *T. gondii* infection in 37.21% of the aqueous humor samples, confirming the clinical hypothesis of toxoplasmic active focal necrotizing retinochoroiditis. A previous study reported that aqueous PCR analyses are useful in AIDS patients with ocular toxoplasmosis (sensitivity of 75%)<sup>(19)</sup> and demonstrated that the aqueous humor is the best source for identifying *T. gondii* infection. Another study also detected *T. gondii* DNA (38%) by PCR in aqueous humor samples<sup>(16)</sup>.

PCR has been found to detect *T. gondii* DNA more effectively in aqueous humor (25%) than in peripheral blood (5%) samples<sup>(22)</sup>. Recently, a study demonstrated that the sensitivity for blood was poor (4.1%) compared with that for ocular samples (35.9%)<sup>(23)</sup>. These findings corroborate our results, which showed that the ability of PCR to detect *T. gondii* DNA was more sensitive in aqueous humor (37.21%) than in peripheral blood samples (2.33%). Positive results in the peripheral blood were found in only one patient (2.3%), while positive results were found in both peripheral blood and aqueous humor samples in 16.27% of the patients. This finding confirms that qPCR can identify parasites circulating in the peripheral blood of patients. *T. gondii* has been found in the blood of acutely and chronically infected patients with ocular toxoplasmosis<sup>(24)</sup>.

## CONCLUSION

qPCR is a useful tool for detecting *T. gondii* DNA in patients with toxoplasmic active focal necrotizing retinochoroiditis and may help in the establishment of appropriate treatment, with the exception of noninfectious uveitis or infectious uveitis caused by other pathogens.

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