OCULAR PRESENTATIONS AND TOXOPLASMA SEROLOGY

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ABSTRACT: During the period 1996-1998, 134 patients suspected of having ocular toxoplasmosis were seen in the Ophthalmology Clinic of the University Hospital, Kuala Lumpur. Clinical presentations in these patients ranged from poor vision to severe retinal detachment. Of these patients, 72% were confirmed positive for *Toxoplasma gondii* infection by serological methods. Chorioretinitis and vitritis were found to be the most apparent symptoms, both having 100% correlation with serological positivity. This was followed by uveitis, floaters, and retinal detachment with correlation at 78%, 75% and 75%, respectively. However, there was no correlation between level of serotitre and ocular presentations. *(JUMMEC 2000; 2:98-102)*

KEYWORDS: Toxoplasmosis, serology, chorioretinitis, uveitis

Introduction

Toxoplasmosis, the most common parasitic infection is also the most frequent cause of focal retinochoroiditis. Current therapy includes the synergistic combination of pyrimethamine, a dihydrofolate reductase inhibitor and sulphadiazine which is a competitive inhibitor of dihydrofolate. Other drugs that can be used include clindamycin, azithromycin, clarithromycin, dapsone and doxycycline.

Diagnosis of ocular involvement of the disease relies on characteristic ophthalmoscopic appearances while the laboratory method is by serological assessment. Although Toxoplasma serology plays an important role in the assessment for prophylatic treatment against encephalitis in HIV-seropositive patients, the diagnostic value in ocular toxoplasmosis however remains controversial (Grant *et al.*, 1990; Aarons *et al.*, 1996;). Some authors believe that a positive titre indicates nothing more than previous exposure to *Toxoplasma gondii* and rely more on clinical judgement. Other methods (antibody calculation, PCR, determination of specific IgA and antibody avidity) do not appear to provide conclusive results (Garweg *et al.*, 1998). Although other workers (Norose et al., 1996) demonstrated the usefulness of quantitative PCR, it was found to have restricted applicability when applied for routine diagnosis (Garweg *et al.*, 1996).

The typical ocular lesion associated with toxoplasmosis is that of necrotizing retinitis or satellite retinitis or inflammation at the edge of an existing scar. However, it can also present in other forms such as anterior uveitis, pars-planitis, periarteritis, retinal vessel occlusion, scleritis and papillitis which can be confused with other diseases such as cytomegalovirus (Rose, 1991; Elkins *et al.*, 1994; Schnyder, 1995).

With more visual impairments believed to be related to toxoplasma infection, clinical examination should always be supported by laboratory tests for establishing diagnosis. It would be impractical however to send every sample from all visual impairment cases for laboratory diagnosis. Therefore, in deciding whether or not sample should be sent for laboratory test, it would be convenient for the clinicians if some guidelines existed. This study was carried out in the attempt to look for the possibility of establishing a correlation between ocular presentations and toxoplasma serology in hope that such correlation can be used to guide physicians to the need of a laboratory test to confirm the diagnosis of ocular toxoplasmosis.

Materials and Methods

Patients

One hundred and thirty four patients with ocular toxoplasmosis were examined over a 3-year period by the

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ophthalmologists at Ophthalmology Clinic of the University Hospital, Kuala Lumpur, and their findings recorded. Five ml of blood sample was taken from each patient and sent for serological testing.

Antigen preparation for in-house direct ELISA

Frozen *T. gondii* tachyzoites harvested from infected BALB/c mice were thawed and washed in PBS. The parasites were then homogenised (on ice) for half an hour and left undisturbed for another half an hour. The homogenate was then transferred into a fresh 3 ml tube and sonicated for 4 pulses (30 secs/pulse). The material was then centrifuged at 10,000 rpm for 15 mins in a Spinwin microcentrifuge. The supernatant was collected and stored at -20° C until use.

Serological tests

a) 'In-house' direct ELISA

Flat bottom microtitre plates (Nunc, USA) were coated with 50 ml/well at 10 mg/ml of prepared antigen (optimal concentration of antigen used was pre-determined using chequerboard titration). The plates were left overnight at 4° C and washed 3 times with PBS-0.05% Tween 20 (PBS-T) to remove excess antigen. The plates were then tap-dried and 100 ml of 1% BSA-PBS (blocking solution) was added to the wells and left at room temperature for 2 hours. The plates were then washed as described earlier, before 50 ml of test serum (diluted in PBS) was added into each well. After 1 hour of incubation at room temperature, the contents were discarded and the plates washed as before. Alkaline phosphatase conjugated IgG or IgM immunoglobulin (Sigma, USA) was added and the plates were incubated at room temperature for 1 hour. The wash procedure was repeated. After the final wash substrate solution was added (100 ml per well) and the plates were incubated in the dark for 30 minutes at room temperature. The enzymatic reaction was stopped by the addition of 50 ml of 3 M sodium hydroxide to each well. The absorbance was measured at 405 nm using an ELISA reader (Microplate Autoreader EL311SX).

b) Toxo-ISAGA

Human IgM antibodies in serum were detected using Toxo-ISAGA commercial kit (BioMerieux Vitek, Inc). Strips pre-sensitised with anti-human IgM monoclonal antibody were reacted with serum samples (diluted in PBS) at 1:100. The strips were covered with adhesive sheet and incubated at 37° C for 2 hours. The strips were then emptied by inverting the plate and washed once in PBS-T. The strips were immediately emptied and washed twice in PBS-T, followed by a 5-minute wash in PBS. After each wash, the strips were thoroughly cleaned on filter paper without allowing to dry. Antigen diluted at 1:20 in BABS buffer was added to the first well (100 ml) and second well (150 ml). The strips were covered as before and incubated at 37°C overnight in a moist chamber. The result was read by placing the strip at approximately 50 cm above a suitably lit white background.

c) Toxo IgG and IgM ELISA

The kits for these tests were obtained from Veda Lab, France. Microwell plates pre-coated with Toxoplasma antigen were used to detect the presence of human IgG and IgM antibodies in serum. Diluted patient sera were added to the wells and incubated. After washing, horseradish peroxidase-labelled antibodies to human IgG/IgM were added. Substrate tetramethylbenzene (TMB) was then added to the well and incubated. The intensity of the colour was measured at 450 nm (A_{450}) using the ELISA reader (Microplate Autoreader EL311SX). The A_{450} value was proportional to the amount of antibodies present in the sample.

Results

In the three-year (1996-1998) period of study, 134 patients suspected of having ocular toxoplasmosis were examined. Malays were the highest number seen, closely followed by the Chinese. Out of 134 patients, 60% of them were male (Figure 1). Examination performed on the patients showed that there were 57 descriptions of ocular presentations, with blur vision/poor vision being the most frequently encountered. The first ten major ocular presentations are shown in Figure 2. Almost half of these patients presented with more than one ocular presentation; combination from these 57 types (Figure 3). One of the patients showed up to five ocular presentations.

Attempt to look for correlation between the ocular presentation and toxoplasma seropositivity was done in the hope that with such correlation, certain ocular presentation shown by the patient, can be used as an 'indicator' that ocular toxoplasmosis need to be included as one of the differential diagnosis. Based on ocular presentations and toxoplasma seropositivity (detection of immunoglobulin subclass IgG in patients' sera), we found that, out of the ten major presentations listed, five of these presentations showed high degree of correlation with toxoplasma seropositivity. Chorioretinitis and vitritis were found to have 100% correlation, followed by uveitis at 78%, while floaters and retinal detachment were at 75% (Table 1).

Discussion

In practice, the diagnosis of ocular toxoplasmosis is most often based on clinical findings. As the clinical signs can be diverse, the criteria for clinical diagnosis may vary

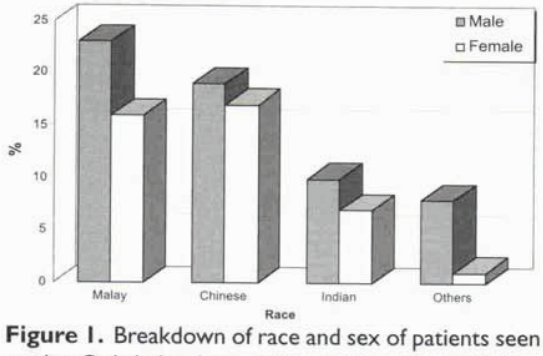

Figure 1. Breakdown of race and sex of patients seen at the Ophthalmology Clinic, University of Malaya Medical Centre, Kuala Lumpur

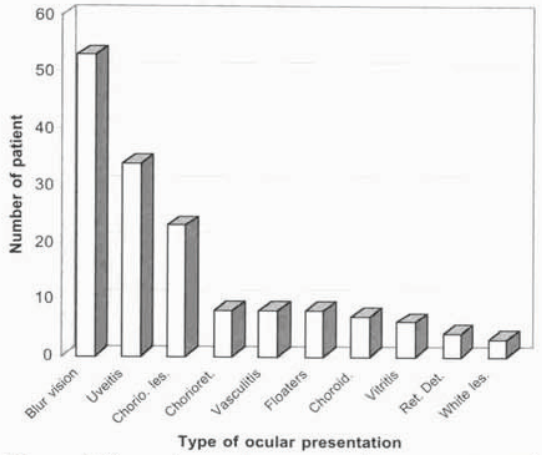

Figure 2. Ten major ocular presentations encountered among patients at the Ophthalmology Clinic, University of Malaya Medical Centre, Kuala Lumpur

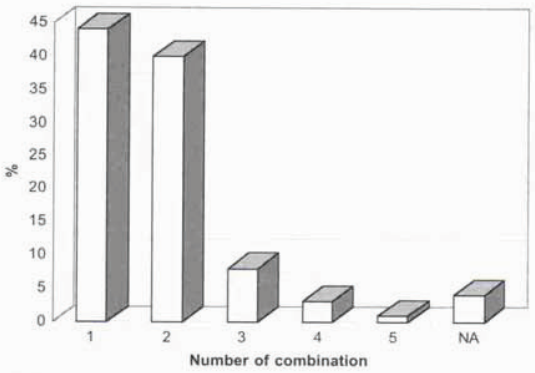

Figure 3. Ocular presentation combinations in patients

from one ophthalmologist to another. The presence of specific antibodies in the serum can therefore be used as supportive evidence of ocular toxoplasmosis in a patient. In this study, we present our findings on the various presentations seen in 134 patients suspected of having ocular toxoplasmosis, and we also tried to establish possible relationship between these presentations and the results of toxoplasma serology.

Fifty-seven different types of presentations (in different combinations) were noted in our study with the more commonly encountered conditions being blurred or poor vision, uveitis and chorioretinal scar/lesion. The number of patients with conditions involving one of these three presentations (single or in combination with other presentations) made up more than half the total number of patients in our study (Table 1). We observed that in certain instances, high frequency of occurrence did not correlate with toxoplasma seropositivity. This is

Table 1. Relationship between ocular presentation and toxoplasmosis

Ocular presentation	Number of patients				
	+ve/+ve	+ve/-ve[a]	-ve/+ve	-ve/-ve	BL
Blur/poor vision	23	44 (67)	1	25	7
Uveitis	13	65 (78)	0	22	0
Chorioretinal scar	13	35 (48)	4	35	13
Chorioretinitis	25	75 (100)	0	0	0
Choroiditis	17	33 (50)	0	50	0
Floaters	25	50 (75)	0	25	0
Vasculitis	0	14 (14)	43	14	29
Vitritis	29	71(100)	0	0	0
White fluffy lesion	0	33 (33)	0	0	67
Retinal detachment	25	50 (75)	0	25	0

[a]Figures in brackets indicate total percentage of patients with positive IgG serotitres
+ve/+ve: positive for IgG andIgM
+ve/-ve: positive for IgG only
-ve/+ve: positive for IgM only
-ve/-ve: negative for IgG and IgM
BL: borderline positive

Table 2. Relationship between ocular presentations and with IgG titre.

Ocular presentation	Percentage (%) of patients having IgG titres stated below						
	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096
Blur/poor vision	0	11	6	14	8	3	17
Uveitis	0	13	0	17	13	5	13
Chorioretinal scar	0	25	12	0	7	12	0
Chorioretinitis	0	33	0	0	67	0	0
Choroiditis	25	0	0	0	0	0	0
Floaters	0	0	0	20	0	20	20
Vasculitis	33	0	0	33	0	0	0
Vitritis	50	50	0	0	0	0	0
White fluffy lesion	0	0	0	67	0	33	0
Retinal detachment	0	0	50	0	0	0	0

seen in the case of poor vision and chorioretinal scar, in which only 59% and 56%, respectively, of the patients had positive IgG serotitres. On the other hand, all (100%) patients who presented with chorioretinitis or vitritis were positive for toxoplasma serology. It was also observed that positive serotitres were found in 61% of the patients with uveitis. This is significantly higher than that obtained by Phaik *et al.* (1991), whose survey in Singapore found only 28.7% of patients with uveitis had positive serotitres for toxoplasmosis.

It is generally believed that ocular toxoplasmosis patients have low positive serotitres because the disease is commonly a localised inflammation and is less likely to result in significant rise in serum antibody levels (Scott, 1974; Rothova *et al.*, 1986). Analysis of our data, however, revealed a wide distribution of serotitres, ranging from 1:64 to 1:4096 (Table 2). The high serotitres obtained might possibly be due to proliferation of toxoplasma trophozoites in the retina (Desmont, 1966). Furthermore, there was no clear link between severity of disease and serotitre levels. This finding is thus in agreement with the study of Damms *et al.* (1991).

Although there are doubts to the diagnostic value of serology in ocular toxoplasmosis, there is still a great body of evidence which advocates the use of serology in the diagnosis ocular toxoplasmosis. Damms *et al.* (1991) showed that the incidence of positive titers and antibody levels were significantly higher in patients with ocular toxoplasmosis while Phaik *et al.* (1991) reported that 89.6% of their clinically positive ocular toxoplasmosis cases had raised serotitres, ranging from 1:64 to 1:4096. Payeur *et al.* (1988) found high correlation between clinical indicators and ELISA test results. The sensitivities of such commercial ELISA kits have been evaluated in the past and the results were in close agreement with those of reference laboratories (Joynson 1989).

In conclusion, our study shows that there was relationship between toxoplasma seropositivity and the incidence of clinical ocular toxoplasmosis. There was, however, no correlation between the various presentations with serotitre levels, suggesting that it may not be useful as a guide during treatment. Nonetheless, the titres can help the physician where the diagnosis may be equivocal.

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