Anti-Toxoplasma Serotitres in Ocular Toxoplasmosis

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Summary

Serological studies are one of the important diagnostic tools used in the investigation of uveitis. This study was based on anti-toxoplasma serotitres obtained from 80 normal healthy adults and 122 patients investigated for ocular toxoplasmosis over a 42 month period. Fifteen per cent of normal healthy sera were found to have low titres (1:64 to 1:256), while 3.8% had high titres (1:1024 or higher). Thirty five eyes of 29 patients had presumed ocular toxoplasmosis based on clinical features and a typical fundal appearance. 89.6% of clinically positive ocular toxoplasmosis were found to have titres ranging from 1:64 to 1:4096. Among the suspected ocular toxoplasmosis cases with a serotitre more than or equal to 1:256, at least 77% had clinical ocular toxoplasmosis.

Serological surveys in the 1960s have demonstrated that *Toxoplasma gondii* is prevalent in Singapore. About 17.2% of apparently healthy individuals and 41.3% of patients in Singapore suspected clinically to have toxoplasmosis were found to harbour antibodies to *Toxoplasma gondii*¹ using haemagglutination techniques. These findings have subsequently² been confirmed using the indirect immunofluorescence technique. This study analyses the antibody titres and prevalence rates in normals and patients presenting with suspected ocular toxoplasmosis.

Materials and Methods

The study was based on sera from 80 normal healthy adults picked randomly (blood-donors) and 122 patients' sera samples being investigated to diagnose/exclude ocular toxoplasmosis in uveitis patients, over a 42 month period.

An indirect immunofluorescence technique was used to detect the antibodies. Antigen (*Toxoplasma gondii*) was obtained from Behringwerke, West Germany and antigen-coated slides were prepared as per manufacturer's instructions. Screening was commenced at a serum dilution of 1:64. Appropriate negative, positive and conjugate controls were included in each test-run. Test and control sera were incubated on the antigen-coated slides for 30 minutes at 37°. The slides were washed in PBS, briefly air-dried and were treated with goat anti-human globulin/FITC conjugates for a further 30 minutes. After a final wash, the slides were mounted and read under UV-illumination. Samples positive at 1:64 were further diluted 4-fold and retested, until their respective end-points.

Toxoplasma serology was ordered in 122 patients as part of the investigation for uveitis.

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Table 1. *The serotitres of uveitis patients*

Titre	Negative	1/64	1/256	1/1024	1/4096	1/16384	Total
Number	87	7	13	12	1	1	121
Percentage	71.3	5.8	10.7	9.8	1.6	0.8	100

These patients either had chorioretinitis, or features of granulomatous uveitis, recurrent uveitis or severe uveitis.

Those cases which had a typical whitish-yellow, raised, and fuzzy outlined lesion of the retina and choroid, adjacent to a previous chorioretinal scar, associated with vitritis and vasculitis, were labelled 'clinically positive'. An attempt was made to study the correlation between these clinically positive cases and their serotitres.

Results

Of the 80 healthy normal blood samples screened, 18.8% were found to have positive anti-toxoplasma antibodies. Fifteen per cent of the healthy adults had low titres (1:64 to 1:256), while 3.75% had high titres (1:1024 or higher).

Of the 122 patients with uveitis syndromes, positive titres were found in 28.7%, the distribution of titre dilutions were as follows: 1:64–5.8%, 1:256–10.9%, 1:1024–9.8%, 1:4096–1.6%, 1:16384–0.8% (Table I).

Thirty-five eyes of 29 uveitis patients were diagnosed clinically to have ocular toxoplasmosis, six patients having bilateral involvement. This constitutes 23.8% of the total number of patients investigated. Fourteen patients were males and 15 females. The youngest patient was 14 years old, and the

oldest 78 years old. The mean age was 42.9 years.

Figure 1 shows the ethnic group distribution of ocular toxoplasmosis patients compared to the general population. In our series, the majority of patients were Chinese (65.5%), followed by equal numbers of Malays and Indians (10.3%). There was one Thai, one French and two Eurasian patients constituting the remaining 13.8%.

89.6% of clinically positive ocular toxoplasmosis had serotitres ranging from 1:64 to 1:4096. Most of the patients had serotitres of 1:256 and 1:1024 (34.5% and 37.9% respectively) (Figure 2). Two patients were found to have negative serology. Toxoplasmosis was found to involve the right eye in 60% (21 eyes) of cases (Figure 3).

Discussion

Indirect immunofluorescence test has gained wide acceptance as the method of choice in the diagnosis of toxoplasmosis in several routine service laboratories because of the high specificity, sensitivity, and the ease of performance.^{3,4}

The seroprevalence rate estimated for normal healthy persons in this study (18.8%) by the immunofluorescence technique correlates well with that estimated by the indirect

**Ethnic group distributions
Ocular toxoplasmosis vs population**

Fig 1.

**SERO-TITRES OF OCULAR
TOXOPLASMOSIS PATIENTS**

Fig 2.

OCULAR TOXOPLASMOSIS LATERALITY

Fig 3.

haemagglutination method (17.2%).¹ These rates have not changed over the past two decades indicating a steady level of transmission of toxoplasmosis in our population in Singapore.

As most of our patients presented with relapsing toxoplasmic retinochoroiditis which typically does not induce a systemic response,⁵ we relied on the level of a single antibody (IgG) titre in diagnosis.

The normal population serotitre distribution followed a normal curve distribution, but was expectedly negatively skewed. Taking two standard deviations from the mean as the reference point, any serotitre of 1:1024 and above was considered high. Titres lower than 1:1024 were considered to be 'low-positive' and thought to be due to past infection/exposure. The distribution of titres in clinical toxoplasmosis is significantly different from the population sample ($P < 0.001$, Chi-squared test) (see Figure 4).

89.6% of clinically positive ocular toxoplasmosis had serotitres ranging from 1:64 to 1:4096. Most of the patients had serotitres of 1:256 and 1:1024 (34.5% and 37.9% respectively). This differs from other reports which used the dye test as the diagnostic tool. It was found that ocular toxoplasmosis patients had generally low positive serotitres.^{6,7} Ocular toxoplasmosis is commonly a localised inflammation and is less likely to cause a significant rise in antibody levels, unless there is proliferation of toxoplasma trophozoites in the retina.⁵

As 82.8% of the clinical toxoplasmosis patients had serotitres greater than or equal to 1:256, this suggests that any serotitre above this dilution is likely to be significant. Two

patients with clinical ocular toxoplasmosis had negative serotitres.

However, this does not exclude infestation unless negative results are obtained by the ELISA test as this is the most sensitive test currently available.⁸

In our epidemiological study, we found that age was not a predisposing factor. The youngest patient was 14 years old, whilst the oldest was 78 years old. The mean age was 42.9 years.

When compared to the general population statistics obtained in the 1989 population census, the disease was found to occur more commonly in Indians.

Sixty per cent of cases involved the right eye. The predominance of the right eye involvement may be because the right common carotid artery is the first branch of the aortic arch.

Six patients had bilateral disease, their serotitres, the median of which was 1:256, were not markedly elevated compared to unocular cases.

Figure 3 shows the percentage of clinically positive ocular toxoplasmosis patients to patients with uveitis syndromes. It can be seen that there is a definite correlation between the serotitres and the incidence of clinical ocular toxoplasmosis. Hence, among the suspected ocular toxoplasmosis cases with a serotitre more than or equal to 1:256, at least 77% have clinical ocular toxoplasmosis.

Conclusion

This study shows that antitoxoplasma titres in patients with clinical ocular toxoplasmosis are

ANTI-TOXOPLASMA SERO-TITRES Normal adults vs toxoplasmosis patients

Fig 4.

significantly different from a sample of the general population. Seventy-seven per cent of ocular toxoplasmosis patients were found to have a titre of 1:256 or more. Although it has been shown that the indirect immunofluorescence test is not conclusive for ocular toxoplasmosis, it was still useful in our context due to the generally low levels of antibodies in our population.

Key words: ocular toxoplasmosis, anti-toxoplasma serotitres.

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