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An age-adjusted seroprevalence study of Toxoplasma antibody in a Malaysian ophthalmology unit

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ABSTRACT

Toxoplasma gondii is a public health risk in developing countries, especially those located in the tropics. Widespread infection may inflict a substantial burden on state resources, as patients can develop severe neurological defects and ocular diseases that result in lifelong loss of economic independence. We tested sera for IgG antibody from 493 eye patients in Malaysia. Overall age-adjusted seroprevalence was estimated to be 25% (95% CI: [21%, 29%]). We found approximately equal age-adjusted seroprevalence in Chinese (31%; 95% CI: [25%, 38%]) and Malays (29%; 95% CI: [21%, 36%]), followed by Indians (19%; 95% CI: [13%, 25%]). A logistic regression of the odds for *T. gondii* seroprevalence against age, gender, ethnicity and the occurrence of six types of ocular diseases showed that only age and ethnicity were significant predictors. The odds for *T. gondii* seroprevalence were 2.7 (95% CI for OR: [1.9, 4.0]) times higher for a patient twice as old as the other, with ethnicity held constant. In Malays, we estimated the odds for *T. gondii* seroprevalence to be 2.9 (95% CI for OR: [1.8, 4.5]) times higher compared to non-Malays, with age held constant. Previous studies of *T. gondii* seroprevalence in Malaysia did not explicitly adjust for age, rendering comparisons difficult. Our study highlights the need to adopt a more rigorous epidemiological approach in monitoring *T. gondii* seroprevalence in Malaysia.

1. Introduction

Toxoplasmosis is one of the more common parasitic anthropozoonoses worldwide.[1] This inconspicuous disease is caused by *Toxoplasma gondii*, an obligate, intracellular protozoan parasite. Clinical infection with *T. gondii* depends on the immune status of the patient. In immunocompetent patients, almost 90% of the infections are asymptomatic; while the rest exhibit a self-limiting, flu-like illness that does not require any treatment.[2]

From a clinical ophthalmology perspective, *T. gondii* is a well-known cause of posterior granulomatous uveitis in immunocompetent people.

A study in Japan assessed the seroprevalence of anti-Toxoplasma antibodies in various ocular diseases[3] and noted a higher incidence of IgG seropositivity in patients with macular degenerative lesions.

It has been estimated that up to one-third of the world's population may be infected with *T. gondii*.[2] In Malaysia studies have been done since the 1970s to assess the prevalence of latent toxoplasmosis in various patient groups.[4] In a review of the epidemiology of toxoplasmosis in Malaysia,[5] it was reported that samples from diverse sources such as the general healthy population, blood donors, pregnant women and HIV patients yielded a range

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of toxoplasmosis prevalence from10–50%. In addition, the extent of chronic infection in the general healthy population was estimated to range from 14–30%.

As most of the studies were done quite some time ago, there is a paucity of data regarding the current prevalence of *T. gondii* infection in Malaysia. Studies on the associations between toxoplasmosis prevalence and various factors such as the degree of urbanisation, socioeconomic status, occupation and ethnicity are available.[6] However, simultaneous quantitative assessment of the odds for prior toxoplasmosis (as indicated by IgG seropositivity) as a function of age, gender, ethnicity and various ocular diseases has never been done. The present study was carried out to shed light on the dual aspects of prevalence and risk factor assessment. It also aimed to find if there was a higher incidence of IgG seropositivity in patients with age-related macular degeneration (ARMD) as compared to other ocular diseases.

2. Methods and materials

2.1. Patient sampling

The current study is observational and cross-sectional. A total of 493 consecutive patients who attended the ophthalmology clinic at the University of Malaya Medical Centre (UMMC), Kuala Lumpur, from January to June 2010, were recruited. Informed consent was obtained from the patients or guardians if the patient was a minor (below 18 years old). Patients with known history of previous toxoplasmosis (ocular or systemic), unexplained chorioretinal scars, pregnant women and immuno compromised patients were excluded from the study. All patients first underwent a complete ocular examination; subsequently 5 ml of venous blood was collected aseptically. For patients who were undergoing any form of surgery, blood collection was done after general anaesthesia had been administered (especially for children). Patients with uveitis underwent further investigations as deemed necessary by the clinical presentation of each case. The diagnosis of ARMD was made based on the clinical picture and specific investigations such as ocular coherence tomography (OCT), fundus fluorescein angiography (FFA) and indocyanine green angiography (ICG). All blood samples were centrifuged using a micro centrifuge machine at 4000 rpm for 10 minutes. The sera were then isolated and stored at −20 °C for further use.

2.2. IgG antibody detection

Specific IgG antibody against *T. gondii* was determined using a standard commercial enzyme-linked immunoabsorbent assay (ELISA, Trinity Biotech, New York, USA), according to the manufacturer's instructions. A value of >1.1 IU/ml (as per the kits' protocol) was regarded as positive for IgG antibody. Because of resource constraints, repeated measurements were not possible for the majority of the sample. We were, however, able to devote sufficient resources to take two measurements for the first 25 patients. Analysis by the Bland–Altman plot[7] showed good agreement between the two measurements, indicating that taking a single measurement for the rest of the patients was unlikely to lead to misdiagnoses.

2.3. Statistical analysis

We performed age-adjustment for estimate of IgG seroprevalence using the direct standardisation method on the age structure of the Malaysian population in 2010.[8] We used logistic regression to model the relation between the odds of prior Toxoplasma infection and four predicator variables: age (natural log scale), gender, ethnicity and ocular diagnoses of the patients. By stepwise removal of variables that are not significant at the 5% level, we obtained the most parsimonious model. We computed the relevant odds ratios (ORs) and their 95% CIs. To test for association between ARMD status and IgG seropositivity, we used Fisher's exact test. For this test, only patients above 40 years old were used since ARMD primarily occurs in this age group. Statistical analyses were done using R (Version 2.10.1).[9]

3. Results

The mean age of the patients was 61 years (SD 18 years; range 2–90). The gender composition was 250 males (51%) and 243 females (49%), while the ethnic composition was 200 Chinese (41%; mean age 66 years, SD 14, range 11–88), 157 Indians (32%; mean age 60 years, SD 19, range 4–90) and 136 Malays (28%; mean age 55 years, SD 20, range 2–87). The patients presented a wide range of ocular diseases including cataract (n = 151), glaucoma (n = 102), retinal vascular diseases (n = 101), ARMD (n = 90), corneal disorders (n = 10) and miscellaneous disorders (n = 17).

Table 1 shows the count and IgG seropositivity according to three main age groups in Malay, Indian and Chinese ethnic groups. Overall IgG seroprevalence was estimated to be 48% (237/493). After age-adjustment, the estimated overall seroprevalence was 25% (95% CI: [21%, 29%]). Among the three ethnic groups, age-adjusted seroprevalence was highest in Chinese (31%; 95% CI: [25%, 38%]), followed by Malays (29%; 95% CI: [21%, 36%]) and Indians (19%; 95% CI: [13%, 25%]). The pairwise difference in age-adjusted seroprevalence was 2% for the Chinese–Malay comparison (95% CI: [−8%, 12%]), 12% for

Table 1
Count and percentage of IgG seropositivity (in parentheses) according to age group and ethnicity

Age Group (years)	Malay	Indian	Chinese	Total
≤24	3/19 (16%)	1/15 (7%)	2/10 (20%)	6/44 (14%)
25–39	1/7 (14%)	0/6 (0%)	1/4 (25%)	2/17 (12%)
≥40	79/110 (72%)	68/136 (50%)	82/186 (44%)	229/432 (53%)
Overall	83/136 (61%)	69/157 (44%)	85/200 (43%)	237/493 (48%)