

ANTI-TOXOPLASMA ANTIBODIES IN HEALTHY ADULTS AND IN DIFFERENT PATIENT CATEGORIES

T C Mohan, H Abdul Jalil, M Nadarajah, E H Sng

ABSTRACT

This study analyzes the anti-toxoplasma sero-titres and prevalence rates in normal healthy adults and in patients presenting with different symptom-complexes. The study was based on sera from 80 normal healthy adults and 2,185 patient sera samples from 2,032 patients (from various clinics and hospitals in Singapore) being investigated for the diagnosis or exclusion of toxoplasmosis, over a 42-month study period.

About 15% of the healthy adults were found to have low IgG antibody titres (1:64 to 1:256), while 3.8% had high IgG titres (1:1024 or higher). Interestingly, among the patients investigated for toxoplasmosis:

- i) more than 20% of those presenting with lymphadenopathy (usually cervical) had antibody titres more than or equal to 1:1024;*
- ii) more than half of all patients with an antibody titre of 1:4096, and more than three-quarters of all patients with a titre exceeding 1:4096 had presented with lymphadenopathy;*
- iii) about 20% of those presenting with ocular symptoms had low antibody titres of 1:64 or 1:256, whereas 7% had higher titres.*

Malay ($p < 0.01$) and Indian ($p < 0.05$) patients had significantly higher seropositive rates than the Chinese. In particular, the Malays ($p < 0.00001$) and Indians ($p < 0.01$) had significantly higher incidence of low-positive titres (1:64, 1:256); conversely the Chinese patients had a significantly higher ($p < 0.01$) incidence of high-positive titres (1:4096 or higher).

Finally, the epidemiology and clinical profiles of patients presenting with acute toxoplasmic lymphadenitis are contrasted with that of patients presenting with ocular manifestations of congenital toxoplasmosis.

Keywords: *Toxoplasmosis, lymphadenopathy, chorioretinitis, immunofluorescence, still-birth*

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INTRODUCTION

Serological surveys in 1960s have demonstrated that *Toxoplasma gondii* is prevalent in Singapore. Animal surveys then⁽¹⁾ showed an average antibody prevalence rate of about 20.5% with the specific prevalence rates in chickens, cats, pigs and cattle being 4.5%, 20.7%, 27.7% and 35.7%, respectively. 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*⁽²⁾ using haemagglutination techniques. These findings have subsequently⁽³⁾ been confirmed using the indirect immunofluorescence technique. The present study expands on these findings and also analyzes the antibody titres and prevalence rates in patients presenting with different symptom-complexes.

Department of Pathology
Singapore General Hospital
Outram Road
Singapore 0316

T C Mohan, MBBS
Resident

H Abdul Jalil
Laboratory Technician

M Nadarajah, MBBS, DipBact
Consultant

E H Sng, MBBS, FRCPA, AM
Consultant and Head

Correspondence to: Dr T C Mohan
Immunology Graduate Program
Dept of Pathology
Tufts University School of Medicine
136 Harrison Avenue
Boston, MA 02111, USA

MATERIALS AND METHOD

The study was based on sera from 80 normal healthy adults picked randomly (blood-donors) and 2185 patient sera samples of 2032 patients (from various clinics and hospitals in Singapore) being investigated for the diagnosis or exclusion of toxoplasmosis, over a 42-month period. The patients' samples were also identified either as being from "first-timers" (total = 2,032) or from "follow-up cases" (total = 153) undergoing repeat titre evaluations. Thus the "follow-up cases" represent patients who have already been counted once as "first-timers".

An indirect immunofluorescence technique was employed 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°C. The slides were washed in PBS, briefly air-dried and were treated with goat anti-human IgG/FITC conjugates for a further 30 minutes. After a final wash, the slides were mounted and read under UV-illumination. Samples positives at 1:64 were further diluted 4-fold and retested, till their respective end-points. The FITC-conjugates were purchased from Behringwerke, West Germany.

Patients being investigated for the first time for anti-toxoplasma titres were divided into different groups based on their ethnic origin and presenting symptoms. The anti-toxoplasma antibody prevalence rates and titres were determined for each group. A test for difference of proportions of patients with specific serotitres between these groups was carried out and its significance was assessed using the Normal and Student's -t distribution.

RESULTS

Table I shows the distribution of antibody titres in 80 normal healthy adults. Of these samples 18.8% were positive for antibodies. Specifically, 15% of the healthy adults had low titres (1:64 to 1:256), while 3.8% had high titres (1:1024 or higher).

Table I
The seroprevalence of anti-*Toxoplasma* antibodies in 80 healthy blood donors, as assessed by immunofluorescence

Titre	No.	%
Negative	65	81.2
1/64	6	7.5
1/256	6	7.5
1/1024	2	2.5
1/4096	1	1.3
1/16384	0	-
Total	80	100

The vast majority of the patients being investigated for toxoplasmosis could be classified into one of these 5 clinical settings:

- presentation to the ENT/surgical department with lymphadenopathy (usually cervical),
- presentation to the Ophthalmology department with ocular symptoms,
- investigation by the O & G department for bad obstetric history,
- investigation by the Paediatric department for suspected congenital toxoplasmosis or
- investigation by the Medical department for prolonged pyrexia.

Table II depicts the antibody prevalence patterns in the patient-samples studied. Interestingly, among the patients investigated for toxoplasmosis:

- more than 20% of those presenting with lymphadenopathy

Table II
Anti-*Toxoplasma* serotitres in patients with different clinical presentations.

Patients' Clinical Presentation	Reciprocals of Titres						Total
	Negative	64	256	1024	4096	16384	
First Timers:							
Lymphadenopathy	176 68.0%	12 4.6%	16 6.2%	23 8.9%	25 9.6%	7 2.7%	259 100%
Ocular symptoms	132 72.1%	11 6.0%	25 13.7%	12 6.6%	2 1.1%	1 0.5%	183 100%
Prolonged fever	116 82.9%	7 5.0%	14 10.0%	3 2.1%	0 0.0%	0 0.0%	140 100%
Abortions	192 84.6%	15 6.6%	13 5.7%	6 2.7%	1 0.4%	0 0.0%	227 100%
Intrauterine death	164 79.2%	12 5.8%	14 6.8%	11 5.3%	6 2.9%	0 0.0%	207 100%
Still-birth	63 75.0%	10 11.9%	7 8.3%	3 3.6%	1 1.2%	0 0.0%	84 100%
IUGR	44 86.3%	4 7.8%	1 2.0%	2 3.9%	0 0.0%	0 0.0%	51 100%
? I.U. Infection	43 84.3%	3 5.9%	4 7.8%	1 2.0%	0 0.0%	0 0.0%	51 100%
? Congenital Toxo	222 87.4%	10 3.9%	15 5.9%	5 2.0%	2 0.8%	0 0.0%	254 100%
Antenatal Screen	70 86.4%	5 6.2%	4 4.9%	2 2.5%	0 0.0%	0 0.0%	81 100%
Others	210	29	36	16	9	1	301
Unknown	157	14	9	12	2	0	194
Subtotal	1589	132	158	96	48	9	2032
Toxo follow-ups	86	13	24	14	13	3	153
Grand Total	1675 76.7%	145 6.6%	182 8.3%	110 5.0%	61 2.8%	12 0.6%	2185 100%

- (usually cervical) had antibody titres more than or equal to 1:1024. This was significantly higher ($p < 0.0001$) than the corresponding percentage among normal adults;
- more than half of all patients with an antibody titre of 1:4096, and more than three-quarters of all patients with a titre exceeding 1:4096 had presented with lymphadenopathy;
 - about 20% of those presenting with ocular symptoms had low antibody titres of 1:64 or 1:256, whereas 7% had higher titres.

The ethnic origin of the patients was studied next. For this analysis, it was ensured that all patients were each only counted once by including only the first-timers (total=2,032). Table III shows the anti-*Toxoplasma* antibody titres detected in the 4 different ethnic groups in Singapore. Malay ($p < 0.01$) and Indian ($p < 0.05$) patients had significantly higher seropositive rates than the Chinese. In particular, as shown in Table IV, the Malays ($p < 0.00001$) and Indians ($p < 0.01$) had significantly higher incidence of low-positive titres (1:64, 1:256); conversely, the Chinese patients had a significantly higher ($p < 0.01$) incidence of high-positive titres (1:4096 and higher).

Table III
Respective numbers and percentages of patients with different toxoplasmosis serotitres in each of the four major ethnic groups in Singapore

Race	Anti-toxoplasma serotitres						Total
	Negative	1/64	1/256	1/1024	1/4096	1/16384	
Chinese	1021 80.3%	64 5.0%	80 6.3%	61 4.8%	40 3.1%	6 0.5%	1272 (100%)
Malays	384 74.6%	48 9.3%	52 10.1%	24 4.6%	5 1.0%	2 0.4%	515 (100%)
Indians	146 75.3%	16 8.2%	21 10.8%	8 4.1%	2 1.0%	1 0.6%	194 (100%)
Others	38 74.5%	4 7.8%	5 9.8%	3 5.9%	1 2.0%	0 0.0%	51 (100%)
Total	1589	132	158	96	48	9	2032

Table IV
Persons of different ethnic groups or with different disease presentations have different patterns of anti-*Toxoplasma* serotitres

Groups compared	Criteria titres	Higher Incidence Group
Chinese vs Malays	1:64 or more	Malays ($p < 0.01$)
Chinese vs Indians	1:64 or more	Indians ($p < 0.05$)
Indians vs Malays	1:64 or more	no significant difference
Chinese vs Malays	1:64 or 1:256	Malays ($p < 0.00001$)
Chinese vs Indians	1:64 or 1:256	Indians ($p < 0.01$)
Indians vs Malays	1:64 or 1:256	no significant difference
Chinese vs Malays	1:4096	Chinese ($p < 0.01$)
Chinese vs Malays	1:4096 or more	Chinese ($p < 0.01$)
Lymphadenopathies vs all patients	1:1024	Lymphadenopathies ($p < 0.005$)
	1:4096	Lymphadenopathies ($p < 0.00001$)
Ocular symptoms vs all patients	1:256	Ocular symptoms ($p < 0.05$)
	1:1024	Ocular symptoms ($p < 0.0001$)

DISCUSSION

A diagnosis of acute toxoplasmosis can be made based on:

- detection of parasites directly in patients' specimens using histological or immunological methods, or
- isolation of the parasite from blood, body fluids or tissues by inoculation of laboratory mice or tissue-culture cells, or
- serological methods for the detection of *Toxoplasma* specific antibodies.

Because of the high specificity and sensitivity, and the ease of performance, only the latter is popularly done in routine diagnostic laboratories. Though several serodiagnostic methods have been employed, the indirect immunofluorescence test^(4,5) has gained wide acceptance as the method of choice in several routine service laboratories. In our laboratory, this technique is routinely used. An IgG antibody titre of 1:1024 or more is considered as "high-positive" whereas titres lower than 1:1024 are considered to be "low-positive" and are thought to be due to past infection/exposure.

The anti-*Toxoplasma* seropositive rate estimated for normal healthy persons in this study (18.8%) by the immunofluorescence technique correlates well with the seroprevalence rate estimated by the indirect haemagglutination method (17.2%) 22 years ago⁽²⁾. These rates have not changed over the past 2 decades indicating a steady level of transmission of *Toxoplasma gondii* in our population in Singapore.

Serological diagnosis of acute toxoplasmosis has classically been made based on the following criteria:

- antigen-specific IgM,
- four-fold rise in antigen specific antibody titres, or
- a single raised value of antigen-specific IgG in the absence of the former 2 data.

In our experience, the incidence of antigen-specific IgM as detected by indirect immunofluorescence is very low. Others⁽⁶⁻⁸⁾ have also found this technique to be difficult because of the high false-negative rates and the narrow time-period within which the raised IgM can be picked up. As there is often a considerable time-gap between disease onset and laboratory investigation, the 4-fold rise in titre is also often missed. Thus in our context, the diagnosis of acute toxoplasmosis rests heavily on a single significantly raised IgG titre (1:1024 or more).

The majority of such patients with significantly high IgG titres had presented with lymphadenopathy. A parallel study⁽⁹⁾ of 34 such patients revealed that the majority of them

- are Chinese,
- aged 21 to 35,
- presented with a painless, mobile, solitary cervical node of 3 to 4 weeks duration as the only symptom,
- had biopsies where the histopathology was suggestive of toxoplasmosis
- were not treated and had no sequelae.

In contrast, patients presenting with ocular symptoms demonstrated lower serotitres as noted in other studies done regionally⁽¹⁰⁾ and elsewhere⁽¹¹⁾. These could represent the adult manifestations of congenital toxoplasmosis in our community.

The higher incidence of anti-*Toxoplasma* antibodies in Malays and Indians compared to the Chinese has previously been recorded in studies conducted locally^(3,12) as well as in Malaysia⁽¹³⁻¹⁵⁾. This has been attributed to the close association of Malays with their pet cats. The high incidence of anti-*Toxoplasma* antibodies in Malays also correlates directly with the incidence of specific pathology attributable to chronic toxoplasmosis. Of all 183 patients investigated for clinically suspected ocular toxoplasmosis, the Malays (Table V) comprised:

- only 11% of all patients eventually shown to be seronegative, but

Table V
The ethnic group composition of 133 *Toxoplasma* seronegative and 50 *Toxoplasma* seropositive patients, clinically suspected to have ocular toxoplasmosis

	Chinese	Malays	Indians	Others	Total
Titre *****					
Negative:	100 (75%)	15 (11%)	13 (10%)	5 (4%)	133 (100%)
1:64 or more:	27 (54%)	16 (32%)	4 (8%)	3 (6%)	50 (100%)
	127	31	17	8	183

- 32% of all patients eventually proven to be seropositive.

Table V also shows that of all Malays investigated for ocular toxoplasmosis, more than 51% were seropositive, whereas the corresponding figure for the Chinese is only 21%. A higher incidence of ocular toxoplasmosis in Malays has also been noted in Malaysia⁽¹⁰⁾. Though the Indians also have a high seropositive rate relative to the Chinese, it is difficult to demonstrate a correspondingly higher incidence of specific pathology because of the smaller numbers.

The significantly higher occurrence of anti-*Toxoplasma* serotitres in excess of 1:1024 among the Chinese (compared to the rest) has also been noted previously⁽³⁾. This could possibly be due to infection transmitted from eating infected pork. It has been established that people who eat improperly cooked pork have raised anti-*Toxoplasma* serotitres⁽¹⁶⁾ and that porcine toxoplasmosis is common in Singapore⁽¹⁷⁾. Thus, pigs in Singapore may act as a reservoir for the transmission of acute toxoplasmosis.

It is tempting to speculate that toxoplasmosis in our adult community is manifesting itself in one of 2 different patterns:

- Acute acquired toxoplasmosis:
The typical patient is a young Chinese presenting with a solitary cervical lymph node; a typical serotitre would be 1:4096. Undercooked or raw pork may be an important factor in disease transmission.
- Adult manifestations of congenital toxoplasmosis:
The typical patient may be a middle-aged or young Malay presenting with blurring of vision and physical findings suggestive of ocular toxoplasmosis; serotitres are typically 1:256 or less. Contact with cats may be important for the transmission of this form of the disease.

Finally, the large number of patients with bad obstetric history and test seropositive (as shown in Table II) call for a systematic prospective study. Though case-reports of infants with congenital toxoplasmosis have been recorded in Singapore⁽¹⁸⁾, the incidence of congenital toxoplasmosis here remains unknown. It is recommended that all expectant females be tested serologically at regular intervals throughout their antenatal follow-up. Such routine testing, if instituted, will help detect all cases of congenital toxoplasmosis as recent advances⁽¹⁹⁾ allow prenatal diagnosis of congenital toxoplasmosis to be reliably made and successfully managed⁽²⁰⁾.

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