

# TOXOPLASMOSIS IN HIV/AIDS PATIENTS IN MALAYSIA

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**Abstract.** Three hundred and one sera of HIV/AIDS patients were tested for anti-*Toxoplasma* IgG antibody by ELISA technique. The seroprevalence of toxoplasmosis was 41.2% (95%CI: 35.5-46.9) in HIV/AIDS patients. The seroprevalence was significantly higher in the Malay (57.9%) than the Chinese (38.7%), followed by the Indian patients (29.6%) ( $p < 0.05$ ). No possible risk factor, such as contact with cats, consumption of uncooked meat, and history of blood transfusions was found to have any significant association with the presence of anti-*Toxoplasma* antibody in the study sample ( $p > 0.05$ ). Multivariate analysis was employed to find any association between *Toxoplasma* seroprevalence and a single subject having single or multiple risk factors. It was found that the association was not statistically significant ( $p > 0.05$ ). Among the HIV/AIDS study samples, 124 (41.2%) samples were found to have positive anti-*Toxoplasma* antibody, the association between the presence of anti-*Toxoplasma* antibody and CD4 cell count was determined but no statistically significant association was found ( $p > 0.05$ ). During the study period, only one case of active CNS toxoplasmosis was registered and the diagnostic criteria included: clinical presentations, CT scan finding, serological evidence of anti-*Toxoplasma* IgG antibody, and response to anti-*Toxoplasma* therapy.

## INTRODUCTION

*Toxoplasma gondii* is an ubiquitous, obligate intracellular protozoan parasite and cosmopolitan zoonosis. Humans may become infected by ingesting oocysts excreted in cat feces (in cat litter or soil) or by ingesting undercooked meat (pork or lamb) containing tissue cysts (Skiest, 2002). Toxoplasmosis refers to the clinical and/or pathological evidence of disease which is asymptomatic (chronic/latent infection) in at least 90% of the population (Wong and Remington, 1994). In the patient with AIDS, toxoplasmic encephalitis (TE) is primarily due to reactivation of latent infection and most commonly involves the brain (Luft and Remington, 1992). Toxoplasmic encephalitis is the most common clinical disease entity due to toxoplasmosis (Luft and Remington, 1992) and is the most frequent cause of focal intracerebral lesions in patients with AIDS (Handler *et al*, 1983; Luft *et al*, 1984; Levy *et al*, 1985; Navia *et al*, 1986; Luft and Remington, 1988). The purposes of this study were to determine the seroprevalence of toxoplasmosis in HIV/AIDS patients, to determine the association between possible risk factors and disease transmission, and to determine the association between CD4 cell count and *Toxoplasma* seroprevalence, in order to gain some

baseline information from which clinical significance may be drawn. Further studies may be indicated or new approaches with HIV/AIDS patients could be implemented.

## MATERIALS AND METHODS

A cross-sectional study was carried out at both the medical out-patients and in-patient wards, Kuala Lumpur Hospital (KLH) from April to July, 2002. In this study, 301 patients who satisfied the eligibility criteria were recruited. Informed consent was obtained prior to enrollment into the study. The inclusion criteria for the study subjects were (1) HIV seropositivity determined by any serological test (ELISA (I, II), CLIA or LA) and confirmed by Western blot assay, (2) patient aged > 14 years, and (3) information collected, including sociodemographic profile, possible risk factors related to toxoplasmosis, clinical history and presenting signs and symptoms relating to toxoplasmosis. The random selection method was employed to obtain eligible HIV-infected patients during the specified study period, and a standardized structured questionnaire was used to collect necessary information, related to the study's variables.

### Serum samples

Approximately 5 ml of venous blood was drawn from all eligible subjects and the collected samples were kept at -20° C for analysis.

### Detection of IgG antibody to *T. gondii*

The standard ELISA commercial kit (Trinity, Ireland) was used in accordance with the manufacturer's

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instructions at the Department of Parasitology, University of Malaya Medical Center. For this study, the IgG result cutoff point was set at > 51 IU as positive.

### Statistical analysis

Data collected and recorded were analyzed using the statistical software SPSS version 10.0. Median and range were used for estimation and description of the quantitative variables where the latter were described by frequency and percentage. The chi-square test or Fisher's exact test was used for testing the association between possible risk factors and disease transmission, also for the association between CD4 cell count and *Toxoplasma* seroprevalence. The Alpha ( $\alpha$ ) value, allowance for type one error, was set at 0.05 and therefore a p-value of less than 0.05 was regarded as statistically significant to reject the null hypothesis.

## RESULTS

The demographic profiles of HIV/AIDS patients are shown in Table 1. The age range was 18 to 78 years, with a median of 36 years. The preponderant age group was 35 to 44 years (37.2%). Subjects were mainly males (74.4%), Chinese (70.8%), and married (55.5%), had secondary level education (56.8%), were non-laborers (42.9%), and resided in Kuala Lumpur (63.1%). The majority of the subjects who participated in this study had a history of antibiotics related to anti-*Toxoplasma* therapy (64.1%).

The seroprevalence of toxoplasmosis in HIV/AIDS patients was 41.2% (95%CI 35.5-46.9). We found only an association between *Toxoplasma* seroprevalence and race, which was significantly higher in the Malays (57.9%) than the Chinese (38.7%), and followed by the Indian (29.6%) ( $p=0.02$ ), as shown in Tables 2 and 3, respectively.

The result of determining the association between various possible risk factors and *Toxoplasma* seroprevalence in HIV/AIDS patients found that there was no significant difference in the seroprevalence of persons with, or without, a history of contact with cats, consumption of uncooked meat or blood transfusion ( $p>0.05$ ). The results were the same when multivariate analysis was applied ( $p>0.05$ ), as shown in Tables 4 and 5, respectively.

Table 6 shows that no significant association between CD4 cell count and positive anti-*Toxoplasma* IgG antibodies was found in this study ( $p>0.05$ ). Among 124 cases with *Toxoplasma* seropositive status, only one active toxoplasmic encephalitis case was registered, as shown in Table 7.

Table 1  
Demographic and baseline characteristics of the study subjects.

Characteristics	HIV/AIDS patients (n=301)
Range	18-78 years
Median age	36 years
<b>Age group (years)</b>	
15-24	10 (3.32%)
25-34	111 (36.9%)
35-44	112 (37.2%)
45-54	39 (13.0%)
55	29 (9.64%)
<b>Sex</b>	
Male	224 (74.4%)
Female	77 (25.6%)
<b>Race</b>	
Malay	57 (18.9%)
Chinese	213 (70.8%)
Indian	27 (9.0%)
Other <sup>a</sup>	4 (1.3%)
<b>Marital status</b>	
Single	134 (44.5%)
Married	167 (55.5%)
<b>Education</b>	
Primary	104 (34.6%)
Secondary	171 (56.8%)
Tertiary	26 (8.6%)
<b>Occupation</b>	
Laborer <sup>b</sup>	67 (22.3%)
Nonlaborer <sup>c</sup>	129 (42.9%)
Other <sup>d</sup>	105 (34.9%)
<b>Present residence</b>	
Kuala Lumpur	190 (63.1%)
Outside K.L.	111 (36.9%)
<b>Past history of drug use</b>	
No drug use	70 (23.3%)
Drug related to ATT	193 (64.1%)
Drug unrelated to ATT	38 (12.6%)

<sup>a</sup>Other: foreigners who were classified as persons with foreign nationality and persons with first and/or family names that were clearly not Malaysian.

<sup>b</sup>Laborer included farmers and manual laborers.

<sup>c</sup>Nonlaborer included government employee, private sector, merchant and house-wife.

<sup>d</sup>Other included unemployed persons and students.

Table 2  
Seroprevalence of toxoplasmosis in HIV/AIDS patients by ELISA.

ELISA	HIV/AIDS patients (%)	95% CI
Positive	124 (41.2)	35.5-46.9
Negative	177 (58.8)	
Total	301	

## DISCUSSION

From this study, the seroprevalence of toxoplasmosis in HIV/AIDS patients was 41.2% (124/301) and still within a compatible range compared with previous studies in Malaysia, for example 21% (Nissapatorn *et al*, 2001; Shamilah *et al*, 2001), and 51.2% (Nissapatorn *et al*, 2003), respectively. However, reports from other studies showed varying prevalence, such as 60% in Bamako (Maiga *et al*, 2001), 44% in Denmark (Garly *et al*, 1997), 15-37%

Table 3  
Seroprevalence of toxoplasmosis in HIV/AIDS patients by demographic profile.

Variables	HIV/AIDS patients		p-value
	Total	ELISA positivity (%)	
<b>Sex</b>			
Male	224	95 (42.4)	0.551
Female	77	29 (37.6)	
<b>Age group (years)</b>			
15-24	10	4 (40.0)	0.834
25-34	111	45 (40.5)	
35-44	112	43 (38.4)	
45-54	39	19 (48.7)	
55	29	13 (44.8)	
<b>Race</b>			
Malay	57	33 (57.9)	0.028
Chinese	213	82 (38.7)	
Indian	27	8 (29.6)	
Other <sup>a</sup>	4	1 (25.0)	
<b>Marital status</b>			
Single	134	57 (42.5)	0.760
Married	167	67 (40.0)	
<b>Education</b>			
Primary	104	40 (38.5)	0.170
Secondary	171	77 (45.0)	
Tertiary	26	7 (24.9)	
<b>Occupation</b>			
Laborer	67	25 (37.3)	0.604
Nonlaborer	129	52 (40.3)	
Other	105	47 (44.8)	
<b>Present residence</b>			
Kuala Lumpur	94	45 (47.9)	0.144
Outside KL	207	79 (38.1)	
<b>Past history of drug use</b>			
No drug use	70	26 (37.1)	0.428
Drug related to ATT	193	79 (40.9)	
Drug unrelated to ATT	38	19 (50.0)	

<sup>a</sup>Other: foreigners who were classified as persons with foreign nationality and persons with first and/or family names that were clearly not Malaysian.

Table 4  
Association between *Toxoplasma* seroprevalence and possible risk factors in HIV/AIDS patients.

Risk factors	HIV/AIDS patients		p-value
	Total	ELISA positivity (%)	
Contact with cat			
Yes	22	8 (36.4)	0.800
No	279	116 (41.6)	
Consumption of uncooked meat			
Yes	71	31 (43.7)	0.730
No	230	93 (40.4)	
Blood transfusion			
Yes	35	17 (48.6)	0.447
No	266	107 (40.2)	

Table 5  
Seroprevalence of toxoplasmosis in relation to various possible risk factors in HIV/AIDS patients.

Possible risk factors	HIV/AIDS patients		p-value
	Total	ELISA positivity (%)	
No risk	189	74 (59.7)	0.432
Contact with cat (R1)	14	4 (3.2)	0.436
Consumption of uncooked meat (R2)	60	27 (21.8)	0.422
Blood transfusion (R3)	25	14 (11.3)	0.112
R1+R2	3	2 (1.6)	0.358
R1+R3	2	1 (0.8)	0.757
R2+R3	5	1 (0.8)	0.402
R1+R2+R3	3	1 (0.8)	0.838
Total	301	124 (41.2)	-

Table 6  
Association between CD4 cell count and *Toxoplasma* seroprevalence in HIV/AIDS patients.

CD4 cell count (cell/mm <sup>3</sup> )	ELISA positivity		p-value
	Total	n (%)	
< 200	118	51 (43.2)	0.894
200-499	133	54 (40.6)	
500	40	16 (40.0)	

in France (Leport and Remington, 1992), 67.8% in India (Meisheri *et al*, 1997), 4% in South Korea (Oh *et al*, 1999), and in Thailand 22.4% and 23.2% (Sukthana *et al*, 2000; Nissapatorn *et al*, 2001), respectively. The differences in seropositivities among previous studies are based on several factors: the geographical distribution of the study sites,

socioeconomic status, risk behaviors of the human population pertaining to the acquisition of *Toxoplasma* infection and the variety of commercial serodiagnostic methods used in each study, each of which demonstrated its own sensitivity and specificity. However, *Toxoplasma gondii* serology should be implemented as part of the routine screening for all HIV-infected patients, and toxoplasmosis chemoprophylaxis should be given to those with positive *Toxoplasma* serology (Milligo *et al*, 2000) in order to prevent the risk of developing the life-threatening secondary reactivation of cerebral toxoplasmosis in association with AIDS (Holliman, 1990).

In this study, the distribution of *Toxoplasma* seroprevalence in these subjects showed higher rates in the later, than the earlier age groups; however, there was no statistical significance (p>0.05). Despite the fact that *Toxoplasma* infection is considered to be acquired in the early years and tends to increase with

Table 7  
Summary of one active toxoplasmic encephalitis case.

Characteristics	Case
Age	29
Sex	Male
Anti-HIV antibody	Positive
Chemoprophylaxis	Co-trimoxazole
Seizure	Tonic-clonic and recurrent
Neurological deficit	Localized
CT scan	Hypodense space occupying lesion with ring enhancement in left parietal region with edema and abscesses
CD4 cell count	22
Anti- <i>Toxoplasma</i> antibody	Positive
Anti- <i>Toxoplasma</i> therapy	Improved

age (up to middle age), those who have more vulnerable life styles become infected, and then declines in the later years. However, stronger evidence of a result similar to the present one is a subject for further study, in order to determine these associations before any reliable conclusions may be drawn.

We found the seroprevalence of toxoplasmosis was significantly higher in the Malays (57.9%) than the Chinese (38.7%), followed by the Indians (29.6%) ( $p < 0.05$ ). This may be explained by the fact that not only are they the major ethnic group in Malaysia, but also their risk behavior favors close contact with cats, which is postulated as the cause of *Toxoplasma* infection (Yahada, 1991).

No significant association between possible risk factors (contact with cats, consumption of uncooked meat, and blood transfusion) and *Toxoplasma* seroprevalence was found in this study ( $p > 0.05$ ). These findings are in agreement with those of previous studies (Wallace *et al*, 1993; Nissapatom *et al*, 2001; 2002). Since it cannot be explained by a single risk factor, multiple risk factors might provide an explanation, therefore, multivariate analysis was performed to obtain the independent effect of these factors on *Toxoplasma* seroprevalence. The results showed no significant association which may probably be due to the limited sample size in each comparison group or other possible risk factors may have an, as yet, undetermined role in *Toxoplasma* infection. Hence, the results should be interpreted with caution, especially the imprecise association, estimation with a reasonably sufficient

sample size based on the prevalence of risk factors in the study population, is suggested for further study.

The association between CD4 cell count and *Toxoplasma* seroprevalence was not significantly different ( $p > 0.05$ ) from previous studies (Nissapatom *et al*, 2001; 2002). This might suggest that subclinical toxoplasmosis had occurred many years before the study period. Therefore, immune status played no significant role in *Toxoplasma* infection. However, we observed that a patient with AIDS who developed secondary reactivation of cerebral toxoplasmosis had a CD4 cell count of 22 cells/mm<sup>3</sup> in this study. This indicates a significant association between a CD4 cell count of  $< 100$  cells/mm<sup>3</sup> and the disease, which has also been documented by other investigators (Renold *et al*, 1992; Richards *et al*, 1995; Mariuz *et al*, 1997). It is thus still feasible to recommend the CD4 cell count as one prognostic marker in determining HIV disease progression, particularly in association with major opportunistic infections. Toxoplasmic encephalitis is a preventable disease when adequate lifelong chemoprophylaxis is used, and thereafter prevents the relapse of a potentially fatal and easily treatable condition (Chaddha *et al*, 1999; Passos *et al*, 2000).

In conclusion, toxoplasmosis is a common opportunistic infection of the central nervous system that causes a major life-threatening condition and public health problem, particularly in patients with AIDS. Unrecognized, unknown and denied risk behaviors for *Toxoplasma* infection can pose major diagnostic and therapeutic challenges to clinicians in this part of the world.

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