

Seroepidemiology of Toxoplasmosis Among HIV-Infected Patients and Healthy Blood Donors

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Summary

A cross-sectional study was carried out in University of Malaya Medical Centre, Kuala Lumpur. Blood samples from 100 HIV-infected patients and 203 Healthy Blood Donors (HBD) were collected and anti-*Toxoplasma* antibodies were detected by using conventional ELISA. The seroprevalence of toxoplasmosis in HIV/AIDS and Healthy Blood Donors were found to be 21% and 28.1% respectively. There was no significant association between the seroprevalence of toxoplasmosis and various possible risk factors i.e. contact with cat, consumption of undercooked meat and history of blood transfusion in both groups. No significant differences between *Toxoplasma* seroprevalence in HIV/AIDS and Healthy Blood Donors in association with presence of single or multiple risk factors were found. The mean CD4 count among HIV/AIDS patients in this study was 202.23 cell/cumm. There was no significant association between CD4 count and seropositivity for *Toxoplasma* antibodies in HIV/AIDS patients.

Introduction

Toxoplasmosis is caused by *Toxoplasma gondii*, an ubiquitous, intracellular protozoan parasite, and cosmopolitan zoonosis. It is estimated to infect about half of the population in the world¹. Most of human infections, however, are mostly asymptomatic but in recent years, with the emergence of the human immunodeficiency virus (HIV), in patients with acquired immunodeficiency syndrome (AIDS), *T. gondii* has been implicated as one of the most important opportunistic pathogens and severe life threatening disease. Cerebral toxoplasmosis is

one of the more frequent opportunistic infections and the most common cause of brain focal lesions complicating the course of AIDS^{2,3}. The aims of this study are to determine the seroprevalence of toxoplasmosis in HIV-infected patients and healthy blood donors (HBD), to determine the association between possible risk factors and disease transmission, and to determine the association between CD4 count and seropositivity for *Toxoplasma* antibodies in order to gain some baseline information for benefit of patients in term of diagnostic purposes and epidemiological point of view.

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Materials and Methods

Informed consent was obtained from the patients prior to the commencement of the study. Questionnaires were recorded from 100 HIV-infected patients and 203 Healthy Blood Donors with age more than 14 years in similarity regarding to the demographic profiles, possible risk factors and in addition to clinical history, signs and symptoms presentations relating to HIV infection were included in HIV-infected patients' record.

Serum samples

The sera were obtained from two population groups as follow:

1. 100 serum samples from HIV-infected patients at both medical outpatient department and inpatient ward who were suspected of toxoplasmosis.
2. 203 serum samples from Healthy Blood Donors were collected from the blood bank in University of Malaya Medical Centre.

Detection of IgG antibody to Toxoplasma gondii

The conventional ELISA test was performed in Department of Parasitology, University of Malaya Medical Centre. From this study, IgG result above the cutoff was considered as positive.

Statistical analysis

The results were analyzed by using the statistical software, SPSS. The data with quantitative variables were estimated by median and range while qualitative variables were estimated by frequency and percentage. This study was analyzed by Chi-square test or Fisher's exact test in comparing the seroprevalence of toxoplasmosis in HIV/AIDS patients and Healthy Blood Donors, to determine the association between possible risk factors and disease transmission and the association between CD4 count and seropositivity for *Toxoplasma* antibodies. The direct standardization was used to adjust the difference in the age composition of the

sample from the two different population. The age-adjusted seroprevalence was then compared using Pearson Chi-square test. P-value of less than 0.05 is regarded as statistically significant.

Results

The demographic profiles of HIV/AIDS patients and Healthy Blood Donors are demonstrated in Table I. There was no significant association between demographic profiles and seropositivity for *Toxoplasma* infection (Table II). From this study, the seroprevalence of toxoplasmosis in HIV/AIDS patients and Healthy Blood Donors were 21% (21/100) and 28.1% (57/203) respectively. There was no statistical difference between these two groups ($P=0.185$) as shown in Table III. Since, the age composition of the samples in the study subjects were different, the overall prevalence must therefore be adjusted to a standardized population before any conclusion could be made. The result in Table IV illustrates that the majority of two population groups were belong to 25 to 34 years and the prevalence of toxoplasmosis in Healthy Blood Donors (41.1%) was higher than HIV/AIDS patients (23.9%) but was not statistically significant.

Table V showed no statistical significant in association between possible risk factors and seroprevalence of toxoplasmosis in HIV/AIDS patients and Healthy Blood Donors in this study. In order to prevent bias due to confounding risk factors in the analysis above, a multivariable analysis was used to compare the prevalence of toxoplasmosis between HIV/AIDS patients and Healthy Blood Donors with single or combined risk factors. The data showed a significant higher in Healthy Blood Donors than HIV/AIDS patients who denied all possible risk factors to *Toxoplasma* infection as shown in Table VI; 31% vs. 16.7% ($p<0.0001$).

The distribution of *Toxoplasma* antibodies in this

study showed that the majority of subjects in both groups had *Toxoplasma* antibodies negative. However, no significant association between HIV/AIDS patients with available CD4 count and seropositivity for *Toxoplasma* antibodies as shown in Table VII.

The most common characteristic of HIV-infected patients in this study were asymptomatic (74%), symptomatic HIV-infected patients (3%) and AIDS-defining illness (23%) respectively. Among 21 cases with *Toxoplasma* seropositive status, only 1 had active toxoplasmic encephalitis.

Table I: Demographic and baseline characteristics of the study subjects

Characteristics	HIV/AIDS group (100)	HBD group (203)	p-value
Age			
Range	20-73 years	18-65 years	0.0001
Mean	34.50 years	31 years	
Sex			
Male	74 (74.0%)	148 (72.9%)	0.840
Female	26 (26.0%)	55 (27.1%)	
Race			
Malay	7 (7.0%)	58 (28.6%)	0.0001
Chinese	77 (77.0%)	106 (52.2%)	
Indian	7 (7.0%)	32 (15.8%)	
Other races	3 (3.0%)	7 (3.5%)	
Foreigner	6 (6.0%)		
Marital Status			
Single	39 (39.0%)	105 (51.7%)	0.037
Married	61 (61.0%)	98 (48.3%)	
Occupation			
Labourer*	28 (28.0%)	21 (10.3%)	0.0001
Non-labourer**	66 (66.0%)	133 (65.5%)	
Unemployed***	6 (6.0%)	49 (24.1%)	
Education			
Primary level	37 (37.0%)	18 (8.9%)	0.0001
Secondary level	48 (48.0%)	76 (37.4%)	
Tertiary level	15 (15.0%)	109 (53.7%)	
Present residence			
Kuala Lumpur	37 (37.0%)	101 (49.8%)	0.05
Outside Kuala Lumpur	63 (63.0%)	102 (50.2%)	
Past history of drug use			
No drug use	26 (26.0%)	180 (88.7%)	0.0001
Drug relating to ATT #	74 (74.0%)	4 (2.0%)	
Drug unrelated to ATT #	0 (0%)	19 (9.3%)	

- Labourer included farmer and manual labourers
 - Non-labourer included government employee, private section, merchant and house wife
 - Others included religious persons and student
- # Anti-Toxoplasma therapy

Table II: The seroprevalence of toxoplasmosis in HIV/AIDS and HBD groups by the demographic characteristics

Variables	HIV/AIDS group (100)		p-value	HBD group (203)		p-value
	Total n (%)	ELISA positivity n (%)		Total n (%)	ELISA positivity n (%)	
Sex						
Male	74 (74.0)	16 (21.6)	0.797	148 (72.9)	42 (28.4)	0.876
Female	26 (26.0)	5 (19.2)		55 (27.1)	15 (27.3)	
Marital status						
Single	39 (39.0)	10 (25.6)	0.362	105 (51.7)	35 (33.3)	0.085
Married	61 (61.0)	11 (18.0)		98 (48.3)	22 (22.5)	
Races						
Malays	4 (4.0)	3 (75.0)	0.170	58 (28.6)	20 (34.5)	0.447
Chinese	63 (63.0)	14 (22.2)		106 (52.2)	26 (44.8)	
Indian	6 (6.0)	1 (16.7)		32 (15.8)	10 (31.3)	
Sabahan & Sarawakian	3 (3.0)	-		7 (3.5)	1 (14.3)	
Foreigner	6 (6.0)	3 (50.0)		-	-	
Occupation						
Labourer	28 (28.0)	3 (10.7)	0.084	21 (10.4)	4 (19.1)	0.238
Non-labourer	66 (66.0)	15 (53.6)		133 (65.5)	35 (26.3)	
Others	6 (6.0)	3 (50.0)		49 (24.1)	18 (36.7)	
Education						
Primary	37 (37.0)	9 (27.3)	0.818	18 (8.9)	4 (22.2)	0.843
Secondary	48 (48.0)	9 (18.8)		76 (37.4)	22 (28.9)	
Tertiary	15 (15.0)	3 (20.0)		109 (53.7)	31 (28.4)	
Past residence						
Kuala Lumpur	37 (3.07)	7 (18.9)	0.695	101 (49.8)	14 (13.9)	0.147
Outsiders	63 (6.0)	14 (22.2)		102 (50.3)	43 (42.2)	
Past history of drug usage						
No drug used	26 (24.0)	8 (30.8)	0.155	180 (88.7)	49 (27.2)	0.568
Drug related ATT	74 (74.0)	13 (17.6)		4 (1.9)	2 (50.0)	
Drug unrelated ATT	-	-		19 (9.4)	6 (31.6)	

Table III: Seroprevalence of toxoplasmosis in HIV/AIDS and Healthy Blood Donors groups as assessed by the conventional ELISA test

ELISA test	HIV/AIDS group	HBD group	p-value
Positive	21 (21.0%)	57 (28.1%)	0.185
Negative	79 (79.0%)	146 (71.9%)	
Total	100	203	

Table IV: Seroprevalence of toxoplasmosis in HIV/AIDS and Healthy Blood Donors' groups by age groups. The total number of subjects examined in the two groups was used as the standardized population

Age group	HIV/AIDS group		HBD group		Standardized population of HIV/AIDS and HBD groups			p-value
	Total n	Positivity n (%)	Total n	Positivity n (%)	Total n	HIV/AIDS (%)	HBD (%)	
15-24	4	0 (0)	54	14 (25.9)	58	0	15.0	0.56
25-34	46	11 (23.9)	73	30 (41.1)	119	28.5	48.9	0.06
35-44	35	8 (22.9)	47	8 (17.0)	82	18.7	14.0	0.51
45-54	5	0 (0)	24	3 (12.5)	29	0	3.6	1.00
≥ 55	10	2 (20)	5	2 (40.0)	15	1.6	6.0	0.41
Total	100	21 (21)	203	57 (28.1)	303	63.6	85.1	0.19

Age-adjusted seroprevalence

21% 28.1%

Table V: The association between possible risk factors and seroprevalence of toxoplasmosis in HIV/AIDS and HBD groups

Risk factors	HIV/AIDS group		p-value	HBD group		p-value
	Total	ELISA positivity n (%)		Total	ELISA positivity n (%)	
Contact with cat						
Yes	94	19 (20.2%)	0.444	181	48 (26.5%)	0.156
No	6	2 (33.3%)		22	9 (40.9%)	
Undercooked meat						
Yes	76	15 (19.7%)	0.239	173	48 (27.8%)	0.800
No	24	6 (25.0%)		30	9 (30.0%)	
Blood transfusion						
Yes	92	18 (19.6%)	0.232	195	57 (29.2%)	0.071
No	8	3 (37.5%)		8	0 (0%)	

Table VI: Seroprevalence of toxoplasmosis in relating to various possible risk factors in HIV/AIDS and Healthy Blood Donors groups

Possible risk factors	HIV/AIDS group		HBD group		p-value
	Total	ELISA positivity n (%)	Total	ELISA positivity n (%)	
No risk	36	6 (16.7%)	116	36 (31.0%)	0.000
Contact with cat (R1)	6	2 (33.3%)	22	9 (40.9%)	0.870
Undercooked meat (R2)	24	6 (25.0%)	30	9 (30.0%)	0.705
Blood transfusion (R3)	8	3 (37.5%)	8	0 (0%)	0.408
R1 + R2	3	2 (66.7%)	4	3 (75.0%)	0.084
R1 + R3	21	0 (0%)	0	0 (0%)	0.841
R2 + R3	1	1 (100%)	1	0 (0%)	0.729
R1 + R2 + R3	1	1 (100%)	0	0 (0%)	0.171
Total	100	21 (21.0%)	203	57 (28.1%)	-

Table VII: The association between CD4 count and seropositivity for *Toxoplasma* antibodies in HIV/AIDS patients

CD4 count	Total No. of HIV/AIDS patients	ELISA positivity n (%)	p-value
≥ 200	25	4 (23.0)	0.835
< 200	38	7 (18.4)	

Discussion

The seroprevalence of toxoplasmosis in HIV/AIDS patients in this study was 21%. Most cases of clinical toxoplasmosis in AIDS result from reactivation of a chronic infection⁴. However, this figure is different from other studies i.e. 51.2% in Malaysia⁵, 15-37% in France⁶, 5.1% in Mexico⁷, 22.4% in Thailand⁸ and 10-40% in USA⁹. The possible explanations for the differences could be due to the geographical variation of the study sites; diagnostic methods employed in each study which may be more or less sensitive than others and the possible risk factors contributing to the acquisition of the infection.

In the study of Healthy Blood Donors, the *Toxoplasma* seropositivity was 28.1% and this was different from the previous studies done in Malaysia i.e. 13.9% in 1973, 27.4% in 1975, 16% in 1977, 20.9% in 1980 and 30.2% in 1984¹⁰. This could be explained that the prevalence of toxoplasmosis in this study subjects are still within the compatible range that need proper guidelines and management in preventing the impending incidence of toxoplasmosis in the years to come. However, we should take into consideration that once healthy persons turn out to be immunocompromised host, toxoplasmosis is definitely not an easy task to deal with particularly in the clinical presentations which is mimicking with other brain diseases.

In term of age distribution in this present study, we find that the highest prevalence of toxoplasmosis in both groups was in the age

group of 25 to 34 years but declined in the prevalence when the patient's age increased. This can be explained that during this period, the person has more vulnerable life style to acquire *Toxoplasma* infection than other age groups, however, no statistical significance between two groups was found. Further studies with larger sample size are needed to clarify the appropriate interpretation of the data.

Analysis of the association between various possible risk factors and *Toxoplasma* seroprevalence in this study showed no significant relationship between contact with cat, consumption of undercooked meat and history of blood transfusion in HIV/AIDS and Healthy Blood Donors. It is interesting to notify that contact with cat had no significant association with *Toxoplasma* infection in both groups. This result is similar to the study from US Naval Hospital, San Diego, where cat ownership or exposure was not related to *Toxoplasma* antibody seroconversion in HIV-infected adults¹¹ as well as one study in Thailand⁸. Since the data need to be justified, we propose to stratify our subjects according to the risk exposure in multi-variable analysis, and surprisingly, there was significant higher in Healthy Blood Donors (31%) without history of all possible risk factors to *Toxoplasma* infection than HIV-infected patients (16.7%) ($p < 0.001$). This could be resolved that other possible risk factors may have and as yet undetermined role to *Toxoplasma* infection or because of the limited sample size that caution is needed in the interpretation of these data.

In the role of CD4 count as one of the prognostic marker in HIV/AIDS patients, we find that there was no significant association between CD4 count < 200 vs. \geq 200 cell/cumm and *Toxoplasma* infection in this study which was similar to the one reported in Thailand⁸. This could be due to the acquisition of the disease which had arisen many years prior to the study period or due to the low sample size and a larger sample study subjects would be more appropriate before any conclusion would be made to determine this association.

In conclusion, toxoplasmosis is the most life-threatening opportunistic parasitic disease and a major health problem worldwide exclusively in HIV/AIDS patients. This study was emphasized on the overall of toxoplasmosis as well as its characteristics which will be seen more frequently in this region.

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