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Seroprevalence of *Toxoplasma gondii* infection among patients in Hospital Universiti Sains Malaysia

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Abstract. Toxoplasmosis is a worldwide endemic zoonotic infection caused by the obligate coccidian parasite *Toxoplasma gondii*. To assess the seroprevalence of *T. gondii* infection among hospital population of Hospital Universiti Sains Malaysia (HUSM), a cross-sectional study was conducted using serum samples of 102 participants. Samples were screened for the presence of anti-Toxoplasma IgG and IgM antibodies by enzyme-linked immunosorbent assay (ELISA). Only one sample recorded as IgM positive (0.98%) (1/102; 95% CI = 0.02–5.34%), whereas 44.12% (45/102; 95% CI = 34.29–54.29%) were IgG positive. The study showed that seroprevalence of toxoplasmosis significantly differs between age groups ($p < 0.001$), with high prevalence of *T. gondii* infection (37.8%) among patients aged less than one month. Moreover, the study indicated a significant difference in the prevalence of toxoplasmosis between males and females. Additionally, a significant dependent relationships were observed ($p = 0.003$), when the reasons of toxoplasma serology request was considered, and the highest prevalence (59.6%) observed in congenital toxoplasmosis. In conclusion, the seroprevalence of toxoplasmosis in our study group was high. The study provides preliminary information about the seroprevalence and epidemiology of *T. gondii* infection among the hospital population in HUSM.

INTRODUCTION

Toxoplasmosis is a highly prevalent zoonotic disease caused by the obligate intracellular protozoan parasite *Toxoplasma gondii* (Dubey, 2009). The capability of this parasite to invade various types of human cells as well as infect virtually all warm-blooded animals highlighted its major medical and veterinary importance (Dubey, 2008 & Silva *et al.*, 2014). Fortunately, primary acquired *T. gondii* infection in immunocompetent individuals occurs usually asymptomatically, and only a few subjects may experience symptoms, such as fever or cervical lymphadenopathy. In addition, nonspecific clinical signs are sometimes associated with several cases (Robert-Gangneux & Darde, 2012). In contrast, toxoplasmosis in immunocompromised patients gained enormous medical importance because it causes severe tissue damage and possibly serious or even fatal complications. Over and above primary invasion during pregnancy might endanger the life of the fetus as well as that of the infected mother (Calderaro *et al.*, 2009). Furthermore, ocular and congenital toxoplasmosis might be also lethal or life-threatening (Juanah *et al.*, 2013).

Nowadays, different methods have been established to detect *T. gondii* antigens or anti *T. gondii* antibodies, including immunological testing, histological identification, parasite isolation in tissue culture, and detection of parasite genes by polymerase chain reaction or by a combination of these techniques (Abdullah, 2010). Among all these methods, serological

diagnosis gives satisfactory result; thus, most clinical investigations rely on it to detect anti *T. gondii* antibodies (Holec-Gąsior, 2013).

In Malaysia, several studies have been conducted to estimate the prevalence of toxoplasmosis among different hosts. Anti-toxoplasma antibodies were found in sera of 14.63%, 35.5%, 14.5%, 9.6%, and 7.9% of ducks, goats, cats, dogs, and local cattle, respectively (Chandrawathani *et al.*, 2008; Puvanesuaran *et al.*, 2012). Nevertheless, data about human toxoplasmosis in Malaysia are limited. Ngui *et al.* (2011) reported that the overall prevalence of *T. gondii* infection was 37% among indigenous communities in Peninsular Malaysia (Ngui *et al.*, 2011), whereas a survey conducted by Normaznah (2004) showed that anti-*T. gondii* antibodies were found in 27.8% of farmers in Gombak District, Selangor (Normaznah *et al.*, 2004).

Despite the exceptionally strong socio-economic and clinical effect of *Toxoplasma* infection, to our knowledge, no previous research on *T. gondii* infection among hospitalized patients in the east coast of Malaysia has been reported. Therefore, the present study was conducted to determine the seroprevalence of toxoplasmosis in Hospital Universiti Sains Malaysia (HUSM), the demographic characteristics of the patients, and the association of serology results to the clinical management of toxoplasmosis.

MATERIAL AND METHOD

Study population

This is a retrospective, hospital-based, cross-sectional study conducted at HUSM in Kelantan, Malaysia. In this study, a total of 102 cases were selected on the basis of request for toxoplasma serology test. Multiple specimens from a subject sent for toxoplasma serology within two weeks from the first request were regarded as one. The demographic data, clinical presentation, results of toxoplasma serology, patient's treatment, and treatment outcome were recorded in data sheets.

Serological tests

The presence of anti *T. gondii* IgG, IgM in serum specimens was detected using Platelia Toxo IgM and IgG ELISA (Bio-Rad, USA). The protocol was performed in accordance with the manufacturers' instructions. For IgM titer, the sample result was expressed by the following ratio: sample OD/cut off OD. Samples with ratio < 0.80 were considered negative; sample with ratio ≥ 0.80 and < 1.00, was considered equivocal for the presence of IgM antibodies, while sample was considered reactive for the presence of IgM antibodies if the ratio ≥ 1.00. Depends on the kit interpretation, IgG results were reported in International Units (IU), sera IgG titer < 6 IU/mL is considered negative for anti-*T. gondii* IgG antibodies, 6–9 IU/mL is considered equivocal, and >9 IU/mL is considered positive. Subsequently, 21 IgG-positive cases were subjected to IgG avidity test by using Platelia Toxo IgG Avidity (Bio-Rad, USA) in accordance with manufacturer's instructions. A low IgG avidity (< 20%) indicates recent infection, whereas high IgG avidity (>30%) excludes recent infection (Lappalainen & Hedman, 2004).

Statistical analysis

All data were statistically analyzed by Stata software version 11. The relative proportions were calculated with confidence interval of 95%. Chi-square was used to assess any possible associations.

RESULT

A total of 102 serum specimens received for serological investigation of *T. gondii* antibodies were used in this study. Of the total, only 1 (0.98%) sample recorded as IgM positive (1/102; 95% CI = 0.02–5.34%), whereas 44.12% (45/102; 95% CI = 34.29–54.29%) were identified as IgG positive. Among the IgG-positive participants, additional analysis with regard to specific age groups indicated significant differences between age groups (p = 0.001), with high prevalence of *T. gondii* infection (37.8 %) in

participants aged less than one month, as shown in Table 1. With regard to gender, the result of this study indicated a significant difference in the prevalence of toxoplasma between males and females; comparably the prevalence in females was significantly higher than that in males, as shown in Table 2.

Furthermore, significant dependent relationships were observed (p = 0.003) when the reasons for toxoplasma serology request was considered. Results showed highest prevalence rate in patients suspected to have congenital toxoplasmosis (59.6%). Out of 45 IgG positive samples, only 21 were included in IgG avidity test, results indicate level of avidity ranged between 30 and 100, with a mean (SD) of 55.7 (21.09) (i.e., high antibody avidity indicating past toxoplasma infection) (Juanah, 2013). Among IgG cases, only 6.7% (3 out of 45 cases) were treated and none of IgG-negative (53 cases) and IgM-negative cases was treated. Additionally, the study found that the outcome also differs significantly among the IgG-positive and IgG-negative patient, this proved by 4 patients from IgG positive group died compared to one in the IgG negative group.

DISCUSSION

Toxoplasmosis possess a great impact on the human health in general, with more serious outcomes in immunocompromised patients (Andiappan *et al.*, 2014). Among the Malaysian population the disease is common and has a high prevalence (Nissapatorn and Abdullah, 2004). The present study showed that the seropositivity of *T. gondii* infection

Table 1. Comparison of demographic data between the IgG positive and negative patients

Variable	No. (%)		P value
	IgG positive	IgG negative	
Age group (years)			
Less than one month old	17 (37.8)	28 (62.2)	0.001
One month – 10	6 (19.4)	25 (80.6)	
11 – 20	4 (100.0)	0.00	
21 – 30	5 (83.3)	1 (16.7)	
31 – 40	6 (85.7)	1 (14.3)	
41-50	6 (85.7)	1 (14.3)	
More than 50	1 (50.0)	1 (50.0)	
Reasons for serology request			
Primary	3 (100.0)	0.00	0.003
Congenital	25 (41.7)	35 (58.3)	
Toxoplasmic encephalitis (TE)	8 (66.7)	4 (33.3)	
Without TE	0.00	1 (100.0)	
Ocular toxoplasmosis	7 (70.0)	3 (30.0)	
Others	2 (12.5)	14 (87.5)	
Treatment			
Treated	3 (100.0)	0.00	0.093
Not treated	42 (44.2)	53 (55.8)	
Outcome			
Well / discharged	9 (52.9)	8 (47.1)	0.195
On follow-up	31 (40.8)	45 (59.2)	
Died	4 (80.0)	1 (20.0)	

Fisher's exact

Table 2. The prevalence of *T. gondii IgG antibodies between males and females* (n = 102)

Variables	Male (n=56) n & % (95% CI)	Female (n=45) n & % (95% CI)	Total n & % (95% CI)	P value
IgG positive	20 (35.7) [23.36, 49.64]	25 (54.3) [39.01, 69.10]	45	0.046
IgG negative	36 (64.3)	21 (45.7)	57	

CI = confidence interval. Pearson Chi-squared was used.

was 44.2%, which were mainly IgG positive cases, and only one IgM-positive case was recorded. Various studies from Malaysia have shown varied rates of Toxoplasmosis depending upon the study population, among indigenous communities in peninsular Malaysia a quite low seropositivity of toxoplasmosis was reported (37%), in which 31%, 1.8 and 4.2% were positive for anti-Toxoplasma IgG, IgM and for both anti-Toxoplasma IgG and IgM antibodies respectively (Ngui, 2011). Similarly; other study was also showed 27.8% of farmers had positive IgG antibody in Selangor (Normaznah, 2004). These findings could be interpreted as the high seropositivity of *T. gondii* infection in this study is probably influenced by the sample population. However, the prevalence rate was lower than that reported in different studies conducted in Malaysia likewise (i.e., 61.1% and 49%) (Nissapatorn *et al.*, 2003; Emelia *et al.*, 2012). In contrast, the prevalence still similar to previous study carried out in Malaysia in which the presence of anti *T. gondii* IgG was (44.9%) (Chan *et al.*, 2008).

Globally, reported sero-prevalence of Toxoplasmaosis varies according to geographical areas and population screened. Detection of anti-*T. gondii* antibodies in our study seems to be very high compared to other studies in different regions of the world, for example, 9.3% were IgG positive among healthy blood donors in Taiwan (Chiang *et al.*, 2012), 24.2% in Slovakia (Studeničová *et al.*, 2006), 28.3% in Thailand (Nissapatorn *et al.*, 2011), and 9.1% in the United Kingdom (Nash *et al.*, 2005).

The variation in the rate of infection could partly be explained by the geographical variation; secondly the difference could be due to uses of different diagnostic methods, that may influence the incidence of the infection owing to the variation of test sensitivity, lastly, other seroprevalence-associated risk factors, such as close contact with cats and consumption of undercooked meat can be also conceded. The high infection rate in our study can be due to different factors, including the study subjects were suspect to have toxoplasmosis based on clinical symptoms and signs. These criteria had increased the probability of the subjects to be *T. gondii* seropositive.

High sero-positivity of anti-*T. gondii* antibodies (41.7%) was observed in the samples screened for congenital toxoplasmosis, however, it is difficult to determine whether this IgG was from the mother or from the baby, this finding indicates high positivity among those populations. This result, in some ways, is correlated with the high prevalence of *T. gondii* infection (37.8%) in participants less than one month of age. Moreover, anti *T. gondii* antibody detection was significantly different between age groups. Recently IgG avidity has been proved to be effective for diagnosis of acute toxoplasmosis (Emelia *et al.*, 2014). Of the 21 samples included in IgG avidity testing, results indicate high avidity (30% to 100%); none of these samples had low avidity index, and this exclude recently acquired infection.

This study further pointed out that serological results influence physician management, and therefore, all treated

patients were IgG positive, and 2 out of them had IgG avidity tested on their samples. Even though a positive IgG individuals may not always be treated. The decision to treat is based on the clinical symptoms, signs and interpretation of the toxoplasma serological result in an individual patient. In addition, the study indicated that the outcome differs significantly among the IgG-positive and IgG-negative patients; none of the patients on treatment died. However, in our study, four patients in the IgG positive group died but we did not explore the underlying disease that has contributed to the death of the participants.

CONCLUSION

The overall seroprevalence of *T. gondii*-specific antibodies in our target group was surprisingly higher compared with previous studies conducted in Malaysia as well as in different regions of the world. The toxoplasma serology, especially a positive IgG avidity, helps physicians decide on the treatment. The outcome of treated seropositive cases in this study was poor.

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