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Seroprevalence of Toxoplasmosis among Hemato-oncology patients in Kelantan

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Abstract. Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii* that is prevalent in humans and animals. This study was aimed to determine the seroprevalence of *T. gondii* infection among hemato-oncology patients and its association with sociodemographic and behavioural characteristics. This cross-sectional study was conducted at the Hospital Universiti Sains Malaysia (USM) involving 56 blood samples from hemato-oncology patients. Anti-*T. gondii* IgG and IgM antibodies and IgG avidity were determined using enzyme-linked immunosorbent assays (ELISA). The association of *T. gondii* exposure, sociodemographic, and behavioural characteristics were assessed by a questionnaire and face-to-face interviews. Twenty-eight (50%) patients were seropositive for *T. gondii* antibodies, where 27 (48.21%) patients were IgG+/IgM- and one patient (1.79%) was IgG+/IgM+ with high avidity index, indicating infection of more than 20 weeks. A univariate analysis showed that age, gender, ethnicity, marital status, educational level, employment status, stem cell transplant, blood transfusion, close contact with cats, water supply, and consumption of undercooked meat were not significantly associated with Toxoplasma seropositivity ($p > 0.05$). Our study has demonstrated, for the first time, the serological evidence of *T. gondii* exposure among hemato-oncology patients in Hospital USM. Our findings indicated that latent toxoplasmosis was relatively prevalence among our patients. Therefore, serological screening tests should be considered for immunocompromised patients as well as the implementation of health education programmes to encourage a healthy lifestyle and the consumption of healthy food among them.

INTRODUCTION

Toxoplasmosis is one of the most common zoonotic diseases caused by the obligate intracellular parasite, *T. gondii*. It affects up to one-third of the world's population (Sarkari *et al.*, 2014; Angal *et al.*, 2016; Sahimin *et al.*, 2017). Humans get infected through the ingestion of food or water that contaminated with oocysts; consumption of raw or undercooked meat; exposure to contaminated soil while gardening, children playing in sandpits or close contact with cats; through placental (vertical transmission from mother to foetus); and accidentally, through organ transplants and blood transfusions (Foroutan-Rad *et al.*, 2016; Abamecha and Awel, 2016; Davami *et al.*, 2015; Sarkari *et al.*, 2014).

Toxoplasmosis might be asymptomatic in immunocompetent individuals, but it may cause severe consequences for those with an impaired immune system such as patients with haematological diseases, human immunodeficiency virus (HIV), as well as in foetus (Lass *et al.*, 2012).

Encephalitis, chorioretinitis, and myocarditis have been reported in immunocompromised patients, and it was associated with severe acute infections or the reactivation of past infections (Sahimin *et al.*, 2017; Sarkari *et al.*, 2014).

Sociodemographic factors such as age, gender, ethnicity, type of disease, residence area, educational level, and occupation have been found to significantly affect Toxoplasma seropositivity (Brandon-Mong *et al.*, 2015). Furthermore, it has been discovered that the consumption of raw or undercooked meat, eating unwashed vegetables or fruits, close contact with cats, poor hygiene and climatic factors were found to contribute in *T. gondii* transmission (Alvarado-Esquivel *et al.*, 2011; Tammam *et al.*, 2013; Avelar *et al.*, 2018; Zemene *et al.*, 2012). As far as is known, there is no scientific data on the association between toxoplasmosis and the socio-demographic and behavioural characteristics of hemato-oncology patients. Since this group is considered as immuno-compromised, data from this study would be helpful for the future management of patients.

MATERIALS AND METHODS

Study design and study population

This cross-sectional study was carried out from August 2017 to September 2018. Power and Sample Size Calculation (PS) software Dupont and Jr. (1990) was used to determine the required sample size. The parameters required for calculation of sample size using PS Software were; level of significant (α) β) = 0.80, assumed P_o = seroprevalence of toxoplasmosis in healthy group in Malaysia was 19.9% and assumed P_1 = immuno-compromised patients (HIV) was 44.4% respectively (Brandon-Mong *et al.*, 2015; Nissapatorn *et al.*, 2005). A total of 56 hemato-oncology patients in Hospital USM were selected according to inclusion and exclusion criteria. Patients above the age of 18 years old with haematological malignancies such as lymphoma, leukaemia, and multiple myeloma, either with or without chemo-therapy or radiotherapy, were included. Patients with polyclonal or monoclonal immunoglobulin such as hepatic disease, acute or chronic infections, autoimmune disease, Plasmacytomas, Waldenstrom macroglobulinaemia or heavy chain disease were excluded from the study.

Sample collection

After consent was obtained, the left-over blood samples were collected from the Haematology Department in Hospital USM. These blood samples were spun at 1500 xg for 10 minutes, and the plasma was obtained and stored at -20°C for further use.

Sociodemographic and behavioural data

The sociodemographic data (age, gender, ethnicity, type of disease, residence area, educational level, occupation) and behavioural data (animal contacts, raw or undercooked meat consumption, unwashed raw vegetables or fruits consumption, washing hands before eating, and type of water supply at home) were obtained through a face-to-face interviews using a questionnaire. Age variable has been divided into two groups for the determination of an association between age group and IgG seropositivity of toxoplasmosis through 2×2 Chi-Square procedure, which fulfilled the assumptions of analysis.

Detection of anti-*T. gondii* IgG and IgM antibodies

Specific IgG and IgM antibodies of the *T. gondii* were determined using Platelia Toxo IgG and Platelia Toxo IgM ELISA kits (BioRad, USA) according to the manu-facturer's instructions.

A titre of IgG anti-*T. gondii* $\geq$ 9 IU/ml was considered as positive, and a titre of IgG anti-*T. gondii* < 6 IU/ml was considered as negative. The positive samples indicate latent or pre-existing *T. gondii* infection. For IgM-ELISA assay, sample ratio $\geq$ 1.0 was considered as reactive for the presence of anti-*T. gondii* IgM, while a ratio of < 0.8 was considered as non-reactive. Subsequently, positive IgM and IgG *T. gondii* samples were further tested for IgG avidity using Platelia Toxo IgG-avidity ELISA (BioRad, USA). The results were interpreted according to the

manufacturer's instructions. An avidity index (AI) of < 0.40 was considered as low avidity index and indicate recent infection of less than 20 weeks, while an AI of ≥ 0.50 indicated as past infection of more than 20 weeks.

Statistical analysis

The data was analysed using Statistical Package for the Social Sciences (IBM SPSS, 22.0 Version) software. Descriptive statistics were used to summarize all the data. The association between risk factors were evaluated using Chi-square assay and the *p*-value of less than 0.05 was considered as statistically significant.

Ethical aspects

This study was approved by the Research Ethics Committee (Human), Universiti Sains Malaysia (USM/JEPeM/17030165). All patients were explained verbally about the purpose and procedures of the study, and a written informed consent was obtained prior to the sample taking.

RESULTS

A total of 28 (50.0%) hemato-oncology patients were seropositive for *T. gondii* antibodies. Twenty-seven of these patients were IgG+/IgM- and one patient was IgG+/IgM+ with high avidity index. The socio-demographic and behavioural characteristics of these patients are described in Table 1. It was noted that the seropositivity rate was higher in; patients above 30 years old (78.6%), male (67.9%), Malay (96.4%), married patients (82.1%) higher educational level (67.9%), unemployed group (57.1%), and patients without history of blood transfusion (64.3%) or stem cell transplant (96.4%). Meanwhile, based on the behavioural characteristics, a high seropositivity rate was found in patients who used treated water supply (71.4%) and patients who did not consume undercooked meat (67.9%). Those patients with or without close contact with cats showed the same seropositivity rate (50.0%).

However, a univariate analysis showed that age [$\chi 2$ (1, N=56) = 0.00, $p > 0.05$], gender [$\chi 2$ (1, N=56) = 1.198, $p > 0.05$], ethnicity $p = 0.99$, marital status [$\chi 2$ (1, N=56) = 0.424, $p > 0.05$], educational level [$\chi 2$ (1, N=56) = 0.820, $p > 0.05$], employment status [$\chi 2$ (1, N=56) = 0.354, $p > 0.05$], stem cell transplant [$p > 1.00$], history of blood transfusion [$\chi 2$ (1, N=56) = 0.664, $p > 0.05$], close contact with cats [$\chi 2$ (1, N=56) = 0.072, $p > 0.05$)], water supply [$\chi 2$ (1, N=56) = 0.091, $p > 0.05$], and consumption of undercooked meat [$\chi 2$ (1, N=56) = 0.084, $p > 0.05$] were not significantly associated with toxoplasma seropositivity rate.

The odds ratios that related to Toxoplasma seropositivity were calculated based on sociodemographic data and behavioral characteristic. The odds ratios for males (OR=1.27), Malay (OR=0.96), singles (OR=1.10), those with a low educational level (OR=1.50), unemployed group (OR=1.14), patients without history of blood transfusion (OR=1.20) and patients with stem cell transplant (OR=1.04). According to the behavioural characteristics, the odds ratios for patients who had close contact with cats (OR=1.08), who used untreated water (OR=1.14) and patients who consumed undercooked meat (OR=1.13) showed that they had a greater risk to get infected with *T. gondii*.

DISCUSSION

Toxoplasmosis has a huge impact on public health concern. It is a well known fact that the disease can cause serious complications to immunocompromised patients (Andiappan *et al.*, 2014). Many previous studies in Malaysia have reported the prevalence of toxoplasmosis in different target groups such as pregnant women, orang asli, people with close contact to animals, human immunodeficiency virus (HIV) patients and patients who specifically requested for toxoplasmosis screening (Andiappan *et al.*, 2014; Angal *et al.*, 2016; Brandon-Mong *et al.*, 2015; Ngui *et al.*, 2011; Mohamed and Hajissa, 2016). In the present study, specific Toxoplasma IgG, IgM and IgG avidity levels in hemato-oncology patients were analysed using ELISA, which is one of the standard methods to detect the presence of specific

Table 1. Sociodemographic and behavioural data of hemato-oncology patients

Characteristics	Frequency (%)	Serology		*p* value	OR
		Positive Toxoplasma IgG n (%)	Negative Toxoplasma IgG n (%)		
Age					
18 – 29	21	6 (21.4)	6 (21.4)	1.00	1.00
≥ 30	79	22 (78.6)	22 (78.6)		1.00
Gender					
Male	61	19 (67.9)	15 (53.6)	0.27	1.27
Female	39	9 (32.1)	13 (46.4)		0.69
Ethnicity					
Malay	98	27 (96.4)	28 (50.0)	0.31	0.96
Chinese	2	1 (3.6)	0		
Marital status					
Married	79	23 (82.1)	21 (75.0)	0.52	0.71
Unmarried	21	5 (17.9)	7 (25.0)		1.10
Educational level					
High school and below	27	9 (32.1)	6 (21.4)	0.37	1.50
College and above	73	19 (67.9)	22 (78.6)		0.86
Employment status					
Unemployed	54	16 (57.1)	14 (50.0)	0.59	1.14
Employed	46	12 (42.9)	14 (50.0)		0.86
Blood transfusion					
Yes	41	10 (35.7)	13 (46.4)	0.42	0.77
No	59	18 (64.3)	15 (53.6)		1.20
Stem cell transplant					
Yes	5	1 (3.6)	2 (7.14)	1.00	0.50
No	95	27 96.4)	26 (92.9)		1.04
Close contact with cats					
Yes	48	14 (50.0)	13 (46.4)	0.79	1.08
No	52	14 (50.0)	15 (53.6)		0.93
Water supply					
Pipe	73	20 (71.4)	21 (75.0)	0.76	0.95
Others	27	8 (28.6)	7 (25.0)		1.14
Consumption of undercooked meat					
Yes	30	9 (32.1)	8 (28.6)	0.77	1.13
No	70	19 (67.9)	20 (71.4)		0.95

antibodies. To the best of our knowledge, this is the first study to report on seroprevalence of toxoplasmosis among hemato-oncology patients and its association with socio-demographic and behavioural characteristics of seropositive patients.

In the present study, the seroprevalence rate among these patients was 50.0%. This result was similar with previous studies in Malaysia which reported the seroprevalence rate between 44.8% to 51.2% in HIV and AIDS patients (Daryani *et al.*, 2011). The estimated prevalence of *T. gondii* infection around the world ranges from 26.0% to 42.1% among cancer patients, HIV/AIDS patients and transplant patients respectively (Wang *et al.*, 2017). In previous studies, a low educational level, employment status, blood transfusion, close contact with cats, clean water supply, eating undercooked meat and poor hygiene were among the established risk factors for *T. gondii* infection. In this study, all the

associated risk factors were found to be not significant among our patients. This could be due to environmental factors; climatic conditions, geographical status, disease control and treatment, regional and ethnic customs, and human activities. Based on a previous study, environmental alterations can affect the prevalence, transmission and distribution of the *T. gondii* (Yan *et al.*, 2016).

The seropositivity rate was found to be higher among patients above 30 years old. This result was consistent with previous studies, which found high seropositivity of toxoplasmosis among patients above 30 years old (Zemene *et al.*, 2012; Alvarado-Esquivel *et al.*, 2016; Sarkari *et al.*, 2014). High seroprevalence and greater risk among patients who aged 30 years old and above could be due to longer exposure time towards the *T. gondii* infection, which make them more susceptible (Avelar *et al.*, 2018). Furthermore, with a weakened immune system, older patients are at a higher risk to get infected. Some studies reported that seropositivity rate was increased with age but not significantly associated (Chiang *et al.*, 2012; Minbaeva *et al.*, 2013; Daryani *et al.*, 2011; Davami *et al.*, 2015; Tegegne *et al.*, 2016; Elhence *et al.*, 2010). Our study showed that the seropositivity rate was higher in male patients. This could be due to the fact that males are more active in outdoor activities, sports or work in which they are exposed to contaminated soil (Brandon-Mong *et al.*, 2015). Males and females play different roles, and in male-dominated jobs, such as gardeners and butchers will expose them for a longer periods to contaminated meat as well as contaminated environment (Retmanasari *et al.*, 2017). Other studies by Davami *et al.* (2015) and Minbaeva *et al.* (2013) also reported that gender is not significantly associated with the infection.

With regard to ethnicity, Malays had the highest seropositivity rate and this is similar with previous findings (Brandon-Mong *et al.*, 2015). Majority of our patients in this study were Malays, and this contributed to the high seropositivity rate of toxoplasmosis. Most Malays either keep cats as their pets or there are stray cats around their households, thereby exposing them to the *T. gondii* infection (Brandon-Mong *et al.*, 2015). In the present study, patients with a high educational level had a higher seropositivity rate compared to those with a low educational level, even though this risk factor was not statistically significant. Toxoplasmosis was found to be slightly higher in unemployed group, and this finding was supported by other studies that found no association between seroprevalence rate with educational level and employment status (Tegegne *et al.*, 2016; Avelar *et al.*, 2018; Zemene *et al.*, 2012).

Although it is rare, *T. gondii* can be transmitted through organ transplant and blood transfusion (Alvarado-Esquivel *et al.*, 2016; Sarkari *et al.*, 2014). In this study, 34.71% of seropositive patients had a history of blood transfusion, and one patient (3.57%) had a stem cell transplant. In contrast, other studies have reported a significant association between blood transfusion and toxoplasmosis (Siransy *et al.*, 2016; Sarkari *et al.*, 2014; Alvarado-Esquivel *et al.*, 2011; Zadeh *et al.*, 2013).

Considering the fact that cat is a definitive host for *T. gondii*, close contact with cats might be a major risk factor for its transmission. Twenty-five percent of patients who had close contact with cats were found to be positive with *Toxoplasma* IgG. However, no significant association was noted. This finding was comparable with other studies in this country which concluded that there was no association between having close contact with cats and Toxoplasma infection (Brandon-Mong *et al.*, 2015). Most studies found that there was an association between the handling, preparation or consumption of under-cooked meat with Toxoplasma infection (Iddawela *et al.*, 2017; Sahimin *et al.*, 2017; Walle *et al.*, 2013; Tegegne *et al.*, 2016). Inadequately cooked meat, primarily beef, lamb or chicken, may contain *T. gondii* tissue cysts that can be transmitted when ingested by patients (Hussain *et al.*, 2017). However, the present findings were similar with other studies which found no association between the consumption of raw or under-cooked meat with toxoplasmosis (Avelar *et al.*, 2018; Davami *et al.*, 2015). A previous study

reported that the prevalence of *T. gondii* was higher in individuals who consumed grilled meat (fully-cooked) compared to those who consumed beef broth or stewed meat (Davami *et al.*, 2015).

Toxoplasmosis is considered to be a water-borne disease that occurs in certain places such as in rural and remote areas (Sahimin *et al.*, 2017). *T. gondii* can be transmitted through contamination of water tanks with cat faeces or through water supply that located close to farms where infected cats are around. Significant association between water supply and toxoplasmosis was reported by previous study in Peninsular Malaysia (Sahimin *et al.*, 2017). In contrast, no significant association was found in this study which was similar with the finding from (Brandon-Mong *et al.*, 2015). Furthermore, most of the patients in this study (74.5 %) had a good and proper treated water supply which is from government pipe.

Odd ratios are commonly used in a cross-sectional study to determine whether a particular exposure is a risk factor for a particular outcome (disease or infection) and also to compare the weightiness of various risk factors for the outcome (Szumilas, 2010). In the present study, patients with a low educational level (OR=1.50) had a greater risk to get infected by *T. gondii* compared to the group with a higher educational level (OR=0.86), while the unemployed patients (OR=1.14) had a greater risk to get infected with *T. gondii* compared to employed patients (OR=0.86). This could be due to the lack of knowledge and awareness about the possible infection, improper living conditions, and scarcity or malnourishment which cause them to be more vulnerable to this infection (Siransy *et al.*, 2016). In most parts of the world, malnourishment plays a significant role in the susceptibility and manifestation of severe clinical symptoms of parasitic infections (Bogitsh *et al.*, 2005). With better education and knowledge about the disease, the risk of *T. gondii* transmission can be reduced. Patients without a blood transfusion history (OR=1.20) and without stem cells transplant (OR = 1.04) had a greater risk to get infected with *T. gondii*. Thus, based on our finding we can postulate that stem cells transplant or blood transfusions are not a risk factor in this population for *T. gondii* infection.

In the present study, patients who had close contact with cats (OR=1.08) showed a greater risk of being infected with the *T. gondii*, and this finding is in agreement with a report by (Tegegne *et al.*, 2016). Toxoplasma oocysts may be passed from cats no matter how healthy and protected they may be because they are the first source of this parasite. Those patients who consumed raw or under-cooked meat had a bigger risk (OR=1.13) of being infected with *T. gondii* than patients who did not consume raw or undercooked meat (OR=0.95). This could be due to the possibility of tissue cysts in meat to be accidentally ingested during handling or even during the tasting of cooking (Iddawela *et al.*, 2017). In the present study, it was found that patients who used untreated water (from wells, rivers or other sources) had a greater risk (OR=1.14) to get infected with *T. gondii* compared to patients who used treated water supply (OR=0.95). Possible contamination can occur without a proper water supply, thus increasing the risk of *T. gondii* infection, as it has been found in a previous study that *T. gondii* is one of the water-borne disease (Sahimin *et al.*, 2017).

CONCLUSION

In conclusion, the seropositivity rate of toxoplasmosis was found to be 50.0% among hemato-oncology patients. With an impaired immune system, they might suffer with severe consequences if the infection reactivated. None of the socio-demographic and risk factors in this study were found to be statistically associated with *T. gondii* seropositivity. To the best of our knowledge, this is the first report that provides serological evidence of *T. gondii* exposure among hemato-oncology patients. With seroprevalence (50.0%) of *T. gondii* infection, especially among immunocompromised group, health education programmes should be conducted by public health authorities to

educate and raise awareness regarding the importance of knowledge about the disease and its related complications, especially for those in immunocompromised group.

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Conflict of interest

All authors report no conflict of interest to this study.

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