**Toxoplasma gondii – Prevalence and Risk Factors in HIV-infected Patients from Songklanagarind Hospital, Southern Thailand**

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Toxoplasmosis is one of the most common opportunistic parasitic diseases in patients living with HIV/AIDS. This study aimed to determine the seroprevalence of *Toxoplasma* infection in HIV-infected patients and to identify associated risk factors in *Toxoplasma* seropositive patients. This study was conducted at a regional public hospital in Hat Yai, southern Thailand during October 2009 to June 2010. Blood samples were collected from 300 HIV-infected patients. Each subject also answered a socio-demographic and risk factors associated with *Toxoplasma* infection. The prevalence of anti-*Toxoplasma* IgG antibodies in HIV-infected patients was 109 (36.3%), of which 83 (76.2%) had past infection and 26 (23.9%) had recently acquired *Toxoplasma* infection as indicated by their IgG avidity. Multivariate analysis using logistic regression showed that gender difference (adjusted OR = 1.69, 95% CI = 1.05–2.72) was the only factor associated with *Toxoplasma* infection. From the results obtained, these HIV-infected patients could be at high risk of developing clinical evidence of severe toxoplasmosis. Therefore, it is necessary to introduce primary behavioral practices to prevent *Toxoplasma* infection among HIV-infected patients.

**Keywords:** HIV, IgG avidity, seroprevalence, risk factors, toxoplasmosis

**INTRODUCTION**

Toxoplasmosis is a clinical and/or pathological evidence of a disease caused by *Toxoplasma gondii*, an obligate intracellular protozoan parasite. *Toxoplasma* infection affects about one-third of the world population but the majority of infected individuals are asymptomatic (Montoya and Liesenfeld, 2004). In people who are living with HIV/AIDS, there is an increased risk of reactivation of latent *Toxoplasma* infection in several organs, particularly in the brain leading toxoplasmic encephalitis (TE) that further complicates the course of AIDS (Sukthana, 2006). Globally, the number of patients who died from AIDS has been declining over the years due to the introduction of highly active antiretroviral therapy (HAART). Toxoplasmosis, one of the HIV co-infections, has, however, contributed to the burden of medical care costs (Suwanagool et al., 1997) and to those patients who are repeatedly admitted to government hospitals.
A positive sample for anti-Toxoplasma IgG antibody was also tested for its avidity using a standard ELISA commercial kit (IgG-NovaLisaTM, Dietzenbach, Germany); high avidity (>40%) indicated a past infection while a low avidity (<40%) indicated a recently acquired infection.

Statistical Analysis
Data obtained from both the questionnaire and laboratory tests were entered and analyzed using the statistical software SPSS version 10 (SPSS Inc., Chicago, IL, USA). The data with quantitative variables were expressed as a mean (±SD) and range, whereas, qualitative variables were estimated and presented as frequencies and percentages. The Chi-square ($\chi^2$) test or Fisher exact probability test was chosen to determine the association between possible risk factors and disease transmission. Multivariate analysis adjusted by multiple logistic regressions was used to determine significant differences between demographics or confounding risk factors associated with Toxoplasma infection among study subjects. The $p$-value of $\leq0.05$ was regarded as statistically significant.

RESULTS

Seroprevalence of Toxoplasmosis in HIV/AIDS Patients and in Association with Demographic Characteristics
The seroprevalence of Toxoplasma infection in these patients was 109 (36.3%). Measurement of IgG avidity among Toxoplasma seropositive patients showed 83 (76.2%) had high avidity indicates past infection while 26 (23.9%) patients had low avidity indicates recently acquired Toxoplasma infection.

Using univariate analysis, this study identified that gender and a history of having cerebral toxoplasmosis were statistically significant factors associated with Toxoplasma seropositivity ($p < 0.05$). The data further showed that majority of male patients were significantly found in the age group of 40–59 years, and they make their living through their labor works. It is also shown that these male patients had not received high education, stayed outside the main city and eating of uncooked meat; however, there was no significant association (Table 2). Further analysis using multivariate logistic regression showed that gender (male) plays a significant role in Toxoplasma seropositivity with adjusted odds ratio of 1.69 (95% CI = 1.05–2.72; Table 3).

The Prevalence of TE in Patients with AIDS
The past clinical history of these patients showed that 10/300 (3.3%) of HIV/AIDS patients, aged between 28 and 52 years, 5 males, and 5 females, were diagnosed with TE prior to this
TABLE 1 | Univariate analysis of plausible demographic characteristics, clinical profiles, and other possible risk factors associated with Toxoplasma seropositive HIV-infected patients.

| Demographic characteristics | Number (%) | Number IgG positive (%) | p-Value |
|-----------------------------|------------|-------------------------|---------|
| Age group (years)           |            |                         |         |
| 20–39                       | 150 (50)   | 46 (30.7)               | 0.125a  |
| 40–59                       | 143 (47.7) | 60 (42)                 |         |
| ≥60                         | 7 (2.3)    | 3 (42.9)                |         |
| Sex                         |            |                         |         |
| Male                        | 157 (52.3) | 66 (42)                 | 0.031a  |
| Female                      | 143 (47.7) | 43 (30.1)               |         |
| Marital status              |            |                         |         |
| Single                      | 104 (34.7) | 42 (40.4)               | 0.288a  |
| Married                     | 196 (65.3) | 67 (34.2)               |         |
| Education                   |            |                         |         |
| Primary                     | 75 (25)    | 27 (36)                 | 0.870a  |
| Secondary                   | 153 (51)   | 54 (35.3)               |         |
| Tertiary                    | 72 (24)    | 28 (38.9)               |         |
| Occupation                  |            |                         |         |
| Laborer                     | 174 (58)   | 62 (35.6)               | 0.728a  |
| Non-laborer                 | 59 (19.7)  | 24 (40.7)               |         |
| Otherb                      | 67 (22.3)  | 23 (34.3)               |         |
| Present address             |            |                         |         |
| Songkhla                    | 149 (49.7) | 49 (46.2%)              | 0.378a  |
| Outside                     | 151 (50.3) | 57 (35.8)               |         |
| CD4 (cells/cumm)            |            |                         |         |
| <200                        | 52 (17.3)  | 22 (42.3)               | 0.111a  |
| 200–499                     | 134 (44.7) | 54 (40.3)               |         |
| ≥500                        | 114 (38)   | 33 (29)                 |         |
| History of receiving chemoprophylaxisc | | | |
| Yes                         | 63 (21)    | 24 (38.1)               | 0.744a  |
| No                          | 237 (79)   | 85 (35.9)               |         |
| History of receiving highly active antiretroviral therapy (HAART) | | | |
| Yes                         | 285 (95)   | 100 (35.1)              | 0.051a  |
| No                          | 15 (5)     | 9 (60)                  |         |
| History of toxoplasmic encephalitis (TE) | | | |
| Yes                         | 10 (3.3)   | 7 (70)                  | 0.024d  |
| No                          | 290 (96.7) | 102 (35.2)              |         |
| History of contact with cats | | | |
| Yes                         | 191 (63.7) | 65 (34)                 | 0.272a  |
| No                          | 109 (36.3) | 44 (40.4)               |         |
| History of eating uncooked meat | | | |
| Yes                         | 58 (19.3)  | 20 (34.5)               | 0.744a  |
| No                          | 242 (80.7) | 89 (36.8)               |         |
| History of blood transfusion | | | |
| Yes                         | 4 (1.3)    | 3 (75)                  | 0.106d  |
| No                          | 296 (98.7) | 106 (35.8)              |         |

a p-value was evaluated by χ² test.
b Other includes retiree, unemployed, housewives and students.
c Co-trimoxazole
d p-value was analyzed by Fisher exact probability test.

Our study confirmed statistically significant (p = 0.024) sero-evidence of anti-Toxoplasma (IgG) antibodies in seven cases (data were not shown).

Other Co-opportunistic Infections in HIV/AIDS Patients

During the time of this study, there was no new diagnosis of TE reported from our patients. However, patients with Toxoplasma seropositivity and other concurrent opportunistic diseases were found as follow: 24/72 patients with tuberculosis (TB) had Toxoplasma seropositivity and 18 of these TB patients subsequently developed immune reconstitution inflammatory syndrome (IRIS-TB). Toxoplasma seropositivity was also found in: 15 patients with herpes virus infections (herpes simplex (HS) and herpes zoster (HZ) viruses), 2 patients with cytomegalovirus (CMV) infections, 2 patients with salmonellosis, 3 patients with histoplasmosis, 5 patients with cryptococcal meningitis, 6 patients with penicillosis, and 4 patients with non-tuberculous meningitis (NTM).

Overall, there was no fatal case reported at the end of this study.

DISCUSSION

Approximately half of HIV-infected patients are co-infected with T. gondii (Shimelis et al., 2009; Daryani et al., 2011). In Thailand, previous studies of the prevalence of Toxoplasma infection among HIV-infected patients found the prevalence ranging from 22.4 to 53.7% (Wongkamchai et al., 1995; Chintana et al., 1998; Sukthana et al., 2000; Nissapatorn et al., 2001; Wanachiwanawin et al., 2001). In our study, 36.3% of subjects were infected with Toxoplasma. This is higher than a study among immunocompetent pregnant women (28.3%) in a study conducted in the same location at the same time (Nissapatorn et al., 2011). Differences in the sensitivity of the ELISA test kit may account for the differences in the prevalence of Toxoplasma infection (Chemoh et al., 2013).
IgG avidity testing was developed to avoid the need of conducting confirmatory tests with a second serum sample to determine, if there is a recently acquired infection (Pour Abolghasem et al., 2011). A positive *Toxoplasma* IgG test with a low avidity suggests a recently acquired infection (Liesenfeld et al., 2001; Reis et al., 2006). Based on IgG avidity testing, 8.7% of our subjects had a newly acquired *Toxoplasma* infection and these patients were closely monitored. However, none developed clinical toxoplasmosis during the study.

Using multivariate analysis, male gender was found to be the only significant risk factor for *Toxoplasma* infection. This is consistent with other studies (Nissapatorn et al., 2007; Akanmu et al., 2010). Males were found to be more susceptible to acquire several infections due to their sex steroid hormones that decrease immune responses and influence disease resistance genes and their behaviors (Klein, 2000). These may be the reasons, why the seropositivity of male HIV/AIDS patients to *Toxoplasma* infection was significantly higher than the female HIV/AIDS patients (Roberts et al., 2001). Previously identified risk factors, such as close contact with cats, consumption of uncooked meats and history of receiving a blood transfusion were not significantly associated with *Toxoplasma* infection in our study.

Reactivation of latent *Toxoplasma* infection is common in immunocompromised hosts (Dahnert, 2003) making HIV patients at higher risk for clinical toxoplasmosis. TE is the most common neurological condition (42%) in HIV-infected patients (Ramirez-Crescencio and Velasquez-Perez, 2011). Ten subjects (3.3%) in our study had a previous history of TE as diagnosed by a combination of clinical TE: headaches, seizure, focal neurological deficits, histology and response of therapy, of these 70% (7) were seropositive for *Toxoplasma* infection. This finding is similar to another study who found some of the patients with confirmed TE were not positive for *Toxoplasma* antibodies (Skiest et al., 2000).

**CONCLUSION**

Latent toxoplasmosis is still prevalent in our study population. Gender was the only significant risk factor for *Toxoplasma* infection in our study. Although the

### TABLE 2 | Comparison of plausible demographic characteristics, clinical profiles, and other possible risk factors between male and female HIV-infected patients.

| Demographic characteristics | Number (%) | Male | Female | p-Value |
|-----------------------------|------------|------|--------|---------|
| Age group (years)           |            |      |        |         |
| 20–39                       | 60 (38.2)  | 90 (62.9) | 0.000<sup>a</sup> |
| 40–59                       | 93 (59.2)  | 50 (35)   |        |
| ≥60                         | 4 (2.6)    | 3 (2.1)    |        |
| Marital status              |            |      |        |         |
| Single                      | 47 (29.9)  | 57 (39.9) | 0.092<sup>a</sup> |
| Married                     | 110 (70.1) | 86 (60.1) |        |
| Education                   |            |      |        |         |
| Primary                     | 33 (21)    | 42 (29.4) | 0.248<sup>a</sup> |
| Secondary                   | 84 (53.5)  | 69 (48.2) |        |
| Tertiary                    | 40 (25.5)  | 32 (22.4) |        |
| Occupation                  |            |      |        |         |
| Laborer                     | 103 (65.6) | 71 (49.6) | 0.000<sup>a</sup> |
| Non-laborer                 | 40 (25.5)  | 19 (13.3) |        |
| Other<sup>b</sup>           | 14 (8.9)   | 53 (37.1) |        |
| Present address             |            |      |        |         |
| Songkhla                    | 76 (51)    | 73 (49)   | 0.648<sup>a</sup> |
| Outside                     | 81 (53.6)  | 70 (46.4) |        |
| History of receiving chemoprophylaxis | 40 (25.5) | 23 (16.1) | 0.664<sup>a</sup> |
| No                          | 40 (25.5)  | 120 (83.9) |        |
| Seroprevalence of Toxoplasma infection | 66 (42) | 40 (28)   | 0.011<sup>a</sup> |
| Positive                    | 91 (58)    | 103 (72)  |        |
| Negative                    | 102 (65)   | 89 (62.2) | 0.711<sup>a</sup> |
| History of contact with cats |            |      |        |         |
| Yes                         | 37 (23.6)  | 21 (14.7) | 0.072<sup>a</sup> |
| No                          | 120 (76.4) | 122 (85.3) |        |
| History of eating uncooked meat | 1 (0.6) | 3 (2.1)   | 0.262<sup>c</sup> |
| Yes                         | 156 (99.4) | 140 (97.9) |        |
| No                          | 1 (0.6)    | 3 (2.1)    |        |

<sup>a</sup> p-Value was evaluated by χ² test.

<sup>b</sup> Other includes retiree, unemployed, housewives and students.

<sup>c</sup> p-Value was analyzed by Fisher exact probability test.

### TABLE 3 | Multivariate logistic regression analysis of risk factors associated with *Toxoplasma* seropositivity.

| Demographic characteristics<sup>a</sup> | Adjusted OR (95% CI)<sup>b</sup> | p-Value |
|----------------------------------------|----------------------------------|---------|
| Gender                                 | 1.69 (1.05–2.72)                 | 0.042   |
| History of receiving HAART             | 0.36 (0.13–1.04)                 | 0.095   |
| History of TE                          | 4.30 (1.09–16.99)                | 0.075   |

<sup>a</sup> Only risk factors with a p ≤ 0.10 on univariate analysis were included in multivariate logistic regression analysis.

<sup>b</sup> OR, odds ratio; CI, confidence interval.
number of HIV infected patients in Thailand has decreased nationwide (Ministry of Public Health [MOPH], 2011), we recommend newly infected HIV patients be warned about this opportunistic parasitic disease during the counseling period since both newly acquired and chronic *Toxoplasma* infections in patients with AIDS are occasionally developed clinical toxoplasmosis (Centers for Disease Control and Prevention [CDC], 2009).

**AUTHOR CONTRIBUTIONS**

VN, NS, and PS designed the study. WC, HA, TH, NS, and BC carried out the experiment. WC, NS, and VN helped in manuscript writing and editing. VN, NS, and PS provided opinions and suggestions about this manuscript. All authors read and approved the final version of the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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