Toxoplasma gondii infection: What is the real situation?

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Highlights

• Seroprevalence of Toxoplasma infections in the literature vary greatly.
• Three methods were used to determine Toxoplasma IgG antibodies.
• Seroprevalence rates of Toxoplasma infections vary according to methods used.

Abstract

The prevalence of chronic Toxoplasma infections reported in the literature varies enormously. We hypothesize that one factor could be due to the different methods used in the evaluation of infections. Serological evidence of Toxoplasma infections in 450 pregnant women (PW) and 300 HIV-infected patients (HIV) were investigated by the Sabin–Feldman dye test and two other commercial ELISA kits (kit1 and kit2). Anti-Toxoplasma IgG antibodies obtained from the Sabin–Feldman dye test, ELISA kit1 and ELISA kit2 in the PW subjects were 14.7%, 29.6% and 38.7%, and in the HIV subjects were 13%, 34.7% and 36.3%, respectively. So there were significant differences in the seroprevalences when different diagnostic tests were used (P < 0.05). Regarding Sabin–Feldman dye test as the gold standard for anti-Toxoplasma antibodies detection, we found that the sensitivity and specificity of the ELISA kit1 and kit2 was in the range of their specification. However as the two ELISA kits used in our study identified a much higher prevalence of Toxoplasma infections which indicated that false positive cases were being reported. Based on results obtained, it is therefore highly recommended that research workers should be aware that the reports of serological studies in terms of high positive results should be treated with some skepticism until additional precise diagnostic tools are developed.

1. Introduction

Toxoplasma gondii is an intracellular protozoan parasite of humans and warm blooded animals. It is reported that currently approximately one third of the world’s population has been infected with this parasite (Hill et al., 2005). The majority of human infections result from accidental ingestion of undercooked meat that contains T. gondii tissue cysts or by ingestion of food or water contaminated with oocysts (Bowie et al., 1997). Although, the infection in healthy people is generally asymptomatic and self-limiting, it occasionally results in severe symptoms and even death.
in immunocompromised patients (Luft and Remington, 1992). For example in some HIV-infected patients (HIV), latent *Toxoplasma* infections may be reactivated and lead to cerebral toxoplasmosis especially when their CD4+ cell count decreases below 200 cells/µL (Luft and Remington, 1992; Jones et al., 1999). The clinical manifestations of this disease include an altered mental state, seizures, weakness, cranial nerve disturbances, sensory abnormalities, cerebellar dysfunction, meningismus, movement disorders and neuropsychiatric demonstrations (Luft and Remington, 1992). If a pregnant woman (PW) becomes infected during the first to second trimester this parasite can transmit to the fetus through the placenta and cause neonatal malformations, neurological damage, blindness, or even stillbirth (Fatoohi et al., 2002; Montoya and Liesenfeld, 2004).

The detection of anti-*Toxoplasma* IgG antibodies is the most common method used to identify a person who has been infected with *T. gondii*. The seroprevalence data of *Toxoplasma* infections reported from each area of Thailand differs enormously, i.e., 2.5–28.3% in PW (Morakote et al., 1984; Malee Wong et al., 1989; Chintana, 1991; Chintana et al., 1998; Sukthana, 1999; Tantivanchi et al., 2001; Nissapatorn et al., 2011) whereas the prevalence in HIV patients varied from 22.4% to 53.7% (Wanachiwanawin et al., 2001). Variations in the seroprevalence of *Toxoplasma* infections have been reported to relate to individual subpopulations (Pappas et al., 2009; Flatt and Shetty, 2012), religions (Al-Harthi et al., 2009; Chan et al., 2009) and socioeconomic practices (Alvarado-Esquivel et al., 2012). The various results acquired from the different methods used for detection the seroprevalence have never been questioned. It would be of interest to evaluate whether or not these different methods could be one of the major causative factors for the seroprevalence variations. The serodiagnostic methods routinely used to determine the level of anti-*Toxoplasma* IgG antibodies against *Toxoplasma* infection include the internationally recognized gold standard method, the Sabin–Feldman dye test (Sabin and Feldman, 1948), IFAT (Krainara et al., 2004), IHAT (Contreras et al., 1996), LAT (Mazumder et al., 1988) and the most convenient ELISA method (Wong et al., 1993).

This study aimed to compare the anti-*Toxoplasma* IgG antibodies from 450 PW and 300 HIV subjects when measured by the gold standard Sabin–Feldman dye test and two other available commercial ELISA kits (kit1 and kit2). The seropositivity obtained from the different diagnostic methods were analyzed to determine if it could be one cause of the variations in the seroprevalence results.

### 2. Materials and methods

#### 2.1. Study site and population

Two groups of people at risk, PW and HIV patients who presented at the Songklanagarind Hospital, Hat Yai, Songkhla Province, Thailand were recruited in this study. The study included 300 HIV volunteers in any age group who visited the hospital between October, 2009 and June, 2010 and 450 PW attending the antenatal clinic during the same time as those with HIV. All subjects gave their informed consent and this study was conducted with the approval from the ethical committee of the Faculty of Medicine, Prince of Songkla University, Thailand.

#### 2.2. Collection of blood samples

After informed consent, about 5 mL of venous blood was drawn and serum was collected and kept at −20 °C until tested.

#### 2.3. Detection of anti-*Toxoplasma* IgG antibodies

Each serum sample was screened for anti-*Toxoplasma* IgG antibodies using the gold standard method, Sabin–Feldman dye test (Sabin and Feldman, 1948) and two standard commercial ELISA kits (kit1 and kit2). The Sabin–Feldman dye test was performed at the Department of Protozoology, Faculty of Tropical Medicine, Mahidol University, as the only available laboratory in Thailand. The samples with titers of 1:4 were regarded as positive (Sukthana et al., 2001). For the ELISA assessments, the level of anti-*Toxoplasma* IgG antibodies was obtained and interpreted in accordance with the manufacturer’s instructions. The values of anti-*Toxoplasma* IgG antibodies were considered positive when the ISR (immune status ratio) ≥ 1.10 for the ELISA kit1 and IU (international unit) > 35 IU/mL for the kit2.

#### 2.4. Statistical analysis

Data obtained were analyzed using the Statistical Package for Social Sciences version 17.0 for Windows software (SPSS Inc., Chicago, IL, USA). The data of the seropositivity of *Toxoplasma* infection between PW and HIV, and between different diagnostic procedures were analyzed using the Chi Square test. A P value <0.05 was considered statistically significant.

### 3. Results

The seroprevalence of anti-*Toxoplasma* IgG antibodies in the PW and HIV subjects obtained from each method are shown in Table 1. The prevalence of the *Toxoplasma* seropositive in the PW and HIV subjects were not significantly different when evaluated by the same technique (P > 0.05). However, there were significant differences (P < 0.05) of the seropositive rates when obtained by the different techniques. The highest seropositive rate was obtained from ELISA kit2 (38.7% and 36.3%), followed by ELISA kit1 (29.6% and 34.7%) and the dye test (14.7% and 13.0%), in PW and HIV, respectively. The outcomes of all samples examined by different methods are shown in Table 1.

### Table 1

<table>
<thead>
<tr>
<th>Samples</th>
<th>Number seropositive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dye test</td>
</tr>
<tr>
<td>PW</td>
<td>66 (14.7)</td>
</tr>
<tr>
<td>HIV</td>
<td>39 (13.0)</td>
</tr>
<tr>
<td>Total</td>
<td>105 (14.0)</td>
</tr>
</tbody>
</table>

**Note:** Different lowercase letters in the same row indicate significant differences (P < 0.05).

### Table 2

Frequency of combinations outcome determined by the Sabin–Feldman dye test, ELISA kit1 and ELISA kit2 from 450 pregnant women (PW) and 300 HIV infected patients (HIV).

<table>
<thead>
<tr>
<th>Dye test</th>
<th>Kit1</th>
<th>Kit2</th>
<th>PW</th>
<th>HIV</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>(*)</td>
<td>58</td>
<td>35</td>
<td>93</td>
<td></td>
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<tr>
<td>(+)</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td></td>
<td></td>
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<tr>
<td>(+)</td>
<td>5</td>
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<td>7</td>
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<td>(+)</td>
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<td>93</td>
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<td></td>
</tr>
<tr>
<td>(+)</td>
<td>62</td>
<td>28</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(+)</td>
<td>249</td>
<td>164</td>
<td>413</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(*) Indicated positive result from each method.

(−) Indicated negative result from each method.
are summarized in Table 2. Among the 750 subjects tested, there were only 93 cases (12.4%) showing positive results and 413 (55.1%) showing negative results with all three test methods. Using the Sabin–Feldman dye test as the gold standard for anti-Toxoplasma antibodies detection, we found ELISA kit1 showed 90.5% sensitivity and 78.0% specificity and ELISA kit2 showed 95.2% sensitivity and 71.6% specificity, which were in the range of the specifications from the manufacturer of both kits. However, the ELISA kit1 showed a positive predictive value (PPV) of 40.1%, a negative predictive value (NPV) of 98.1% and the ELISA kit2 showed PPV of 35.3% and NPV of 98.9% by comparison with the dye test. Fig. 1 shows anti-Toxoplasma-IgG antibodies level and the results.

Fig. 1. Comparison of anti-Toxoplasma-IgG antibodies level between ELISA kit1 and kit2, dye test and ELISA kit1 and dye test and ELISA kit2 of 450 PW (A–C) and 300 HIV (D–F), respectively. The cut off for the dye test was 1:4. The dash lines indicate the cut-off value of each ELISA test. The numbers in the table indicate the number of samples that are positive (+) or negative (−) for each test method.
from individual subjects when compared between the two methods. By focusing on both ELISA kits, 107 (23.8%) PW and 79 (26.3%) HIV was found to be positive by both kits, 250 (55.6%) PW and 166 (55.3%) HIV were negative, resulting in a disagreement of 93 (20.67%) from the PW and 55 (18.33%) from the HIV subjects (Fig. 1A and D). This high disagreement was also obtained when comparing the ELISA kit1 and the dye test (Fig. 1B and E) or the ELISA kit2 and the dye test (Fig. 1C and F) being 79 (17.6%) in the PW and 73 (24.3%) in the HIV, and 114 (25.3%) in the PW and 74 (24.7%) in the HIV, respectively.

4. Discussion

Several methods have been employed to elucidate Toxoplasma infections by detecting anti-Toxoplasma IgG antibodies. From a literature review, it seemed that seroprevalence of latent toxoplasmosis varied greatly. Our results strongly support the view that the different methods used played a significant role in the variations of Toxoplasma seropositivity. There have been some studies in Thailand, using the Sabin–Feldman dye test to report that the seroprevalence of Toxoplasma infection in PW was between 7.9% and 13.2% (Chintana et al., 1998; Sukthana et al., 2000; Krainara et al., 2004) and were significantly different from the 21.1% to 23.2% in the HIV cases (Chintana et al., 1998; Sukthana et al., 2000; Nisaaporn et al., 2001). However, our results for PW are similar (14.7%) but we found a much lower Toxoplasma seropositivity in HIV subjects (13%) when compared to earlier studies. The lower seropositivity in HIV subjects from our study may be a result of their primary behavioral practices particularly through consumption of properly cooked foods and clean water in preventing Toxoplasma acquisition before or during the time of their immune deficiency. As a result of their low Toxoplasma prevalent rates, there was no clinically confirmed case of toxoplasmosis in these patients reported during the time of this study. However, this evidence could also be explained by the fact that these patients were given the highly active antiretroviral therapy (HAART) which effectively suppresses the HIV viral load and helps to improve the CD4+ T-cell count followed by reconstitution of some cellular immunity (Pozio, 2004). It has previously been established that T-helper cells (CD4) associated with Toxoplasma infection by encouraging T-cytotoxic cells to directly destroy tachyzoites and activate in B-cell to generate antibodies against T. gondii (Ho-Yen, 1992). This phenomenon may not only prevent secondary reactivation of latent toxoplasmosis but also decrease the susceptibility to other opportunistic infections including those caused by parasites. Most of our HIV subjects (95%) had received HAART over a period of 2–139 months with a median of 57 months (data not shown). Our findings further showed a significant higher overall Toxoplasma seropositivity rate when using an ELISA technique. The data from using commercial ELISA kits to detect the seroprevalence of Toxoplasma infection in Thailand is limited. So far, only two previous studies reported that 53.7% and 5.3% of HIV and non-HIV infected Thai pregnant women, respectively (Wanachiwanawin et al., 2001) and 8.8% of Thai Buddhist monks who lived in the temples (Wiw nitkit et al., 2004) were seropositive for Toxoplasma infection. Our data showed between 29.6–38.7% of PW and 34.7–36.3% of HIV subjects were seropositive when using the ELISA kits. A recent study, using a latex agglutination test reported an exceptionally low number (2.6%) of Toxoplasma infections among women in northeastern Thailand (Sakae et al., 2013) despite the fact that the sensitivity of this test was 93.7–100% (Villard et al., 2012). These variations of seroprevalent rates need further exploration before any conclusions could be made.

Although the dye test is routinely accepted as the reference method for serodiagnosis of human toxoplasmosis, it has a high cost and is unsafe because it needs live tachyzoites as a source of antigens. The ELISA test is one of several serological methods commonly used to diagnose human toxoplasmosis. This assay is low cost, provides quantitative analysis and is convenient to perform. However, our results have indicated clearly that there is a high false positive rate obtained. Furthermore, among the 750 subjects evaluated, we found only 93 (12.4%) that were positive with all three tests used. The majority of these subjects had high levels of IgG antibodies (Fig. 1). The large numbers of false positive samples obtained from the two ELISA kits could be due to the cross-reactivity between Toxoplasma infection and other parasitic disease endemic in this area such as malaria (Abramo et al., 1995). Therefore additional precise diagnostic tools for detecting Toxoplasma infection need to be extensively evaluated. The improvement of the ELISA test in terms of considering the standardization of antigen used may be a significant approach. Currently, a variety of recombinant antigens and proteins are being developed to increase the specificity using an ELISA test or other serological tests. Some of these may choose different antigens for the serodiagnosis of Toxoplasma infections (Kotresha and Noordin, 2010; Selseleh et al., 2012). However, whether these proteins are moving closer to reality for next generation sero-markers of toxoplasmosis, have yet to be seen.

5. Conclusion

Our present study demonstrated that there was no significant difference of Toxoplasma seropositivity between PW and HIV subjects using three different serological diagnostic tests; the Sabin–Feldman dye test as the gold standard test and two commercial ELISA kit1 and kit2. However, when these three different techniques were compared, there were significant differences obtained for Toxoplasma seroprevalence. ELISA kits were most likely shown high false positive for Toxoplasma infection rates. Since, these serological methods are used as a routine or primary screening for Toxoplasma serostatus, therefore, other laboratory investigations such as IgG avidity or molecular analysis should be considered as alternative diagnostic approaches. Based on serological results, clinical evidence of toxoplasmosis is utmost important that clinicians should be aware of to confirm the diagnosis before given a proper treatment.

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