COMPARISON OF INDIRECT IMMUNOFLUORESCENT ANTIBODY TEST AND SABIN-FELDMAN DYE TEST FOR DETECTION OF *TOXOPLASMA GONDII* ANTIBODY IN THAI PREGNANT WOMEN

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Abstract. The aim of this study is to determine the specific IgG antibody for *Toxoplasma gondii* in pregnant Thai women by IFAT, using formalin-fixed whole tachyzoites and comparing the sensitivity and specificity with those of the Sabin-Feldman dye test (SFDT). Blood samples were collected from 189 pregnant Thai women, who came for their first visit at the antenatal clinic of Rajavithi Hospital from March to May 2003. The result of antibody measurement from the indirect fluorescent test showed 66.6% sensitivity and 95.9% specificity, when compared with Sabin-Feldman dye test. IFAT is not time-consuming, easy to perform, and formalin-fixed whole antigens can be kept for a long time. IFAT, therefore, is recommended as a selective test, which can be used in laboratories that cannot perform SFDT.

INTRODUCTION

Toxoplasma gondii, an intracellular protozoan parasite belongs to the Family Eimeriidae. *Toxoplasma* was first discovered in a North African rodent (*Ctenodatylus gundi*) by Nicolles and Manceaux, in 1908. It has been found in many species of mammals and birds worldwide, and also in humans. It has been recognized as a cause of toxoplasmosis. The cat, which is the definitive host, can become infected by ingesting infected rats with cysts. The oocysts excreted by cats are infective to most mammals and birds, which are intermediate hosts, by carnivorism or transplacentally. Human infection occurs commonly from the ingestion of undercooked meat containing the encysted bradyzoits of the parasite, or by ingestion of food or water contaminated with oocysts. Most human infections are asymptomatic, but the fetus may present severe disease if the mother is infected during pregnancy, and in infections of immunocompromized persons. The overall prevalence of this infection is almost 13% of the world population. Various epidemiological studies have shown that the recent data of seroprevalence rate in pregnant Thai women is approximately 13% (Sukthana *et al*, 2000; Tantivanich *et al*, 2001).

The Sabin-Feldman dye test (SFDT) is the gold standard test for toxoplasmosis, but it requires live parasites, and is time-consuming, with complicated procedures and accessory factors; it is not common and easy to offer in a small laboratory. Alternative serological tests, which have been developed, are the latex agglutination test (LAT), indirect hemagglutination test (IHAT), enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody test (IFAT). The dye test, IFAT, IHAT and ELISA are the methods most widely used for diagnosing acute toxoplasmosis. The IFAT technique is highly sensitive, the same as that of the dye test for routine laboratory use (Kelen *et al*, 1962; Sulzer and Hall, 1967). This study determined the specific IgG antibody of *Toxoplasma gondii* in pregnant Thai women by IFAT using formalin-fixed whole tachyzoites, and compared the sensitivity and specificity with those of the Sabin-Feldman dye test.

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MATERIALS AND METHODS

Sample collection

One hundred and eighty-nine blood samples were collected from Rajavithi Hospital between March 2003 and May 2003. The blood was collected at the first visit at the antenatal clinic. The pregnant women's sera were separated from clotted blood by centrifugation and stored at -20˚C until required for testing. IgG antibody to *T. gondii* was detected from all sera by using the indirect fluorescent antibody test and the Sabin-Feldman dye test.

The Sabin-Feldman dye test

The test is based on complement-mediated neutralizing antigen-antibody reaction. The test detects

Toxoplasma IgG antibody in pregnant women's sera reacted with live tachyzoites from the peritoneal fluid of inoculated mice, incubated at 37˚C for one hour; after that, methylene blue is added. Specific antibody in the sample induces complement-mediated cytolysis of the parasites, and the organisms are unable to retain the stain.

Indirect fluorescent antibody test (IFAT)

Antigen preparation. The peritoneal exudate of mice was taken three days after intraperitoneal inoculation of *T.gondii*. One part of exudate was treated with nine parts of 1% formalin in 0.85% NaCl solution for 30 minutes at room temperature. The exudate was centrifuged at 500 rpm for 5 minutes, the supernatant was removed and again centrifuged at 1,500 rpm for 10 minutes. The pellet was resuspended with 10 ml 0.85% NaCl solution and washed twice. The final pellet was resuspended in 1 ml NSS. The concentration of tachyzoites was determined by a hemocytometer count. Stock solution was diluted as necessary with NSS. *Toxoplasma* concentration was approximately 5-6 × 10^3organisms/mm^3. Ten µl *Toxoplasma* tachyzoites in NSS solution was dropped on to each 6-mm diameter of 3 circles on each slide. The slides were air-dried at room temperature and kept at -20˚C until used as antigen.

The test. In this test, killed trophozoites are fixed onto a slide and incubated with dilutions of the tested sample, and using specific fluorescent-labeled conjugates, IgG antibody in tested serum, if present, was detected. Positive samples were determined by complete peripheral or whole parasite. A negative result showed only apical staining. Pregnant women's sera were tested for specific IgG antibody to *T. gondii* by IFAT. Samples were tested using two-fold dilutions, starting at 1:8. Sera reacted with formalin-fixed whole tachyzoites, incubated at 37˚C for 30 minutes, washed in 0.1M PBS, pH 7.2 and air-dried, specific fluorescein conjugates was added and incubated at 37˚C for 30 minutes. After washing again, slides were air-dried and mounted in buffered glycerol. The slides were observed with a fluorescent microscope. End titers were determined by lack of fluorescence.

RESULTS

Staining of *Toxoplasma gondii* with fluorescent-antibody

Toxoplasma covered first with anti-toxoplasma pregnant woman's serum and then with fluorescent-labeled anti-human IgG, showed as positive by a mostly peripheral fluorescence, bright and greenish-yellow color (Fig1a, positive reaction). On the other hand, *Toxoplasma* covered first with normal serum and then with fluorescent-labeled anti-human IgG showed as non-fluorescent staining (Fig 1b, negative reaction).

Sensitivity and specificity of the test

One hundred and eighty-nine of pregnant women sera were tested in parallel by IFAT and the SFDT. The result is shown in Table 1. The IFAT was positive in 8.9% of these cases, ranging from 1:8 to 1:128. Of 189 cases, 7.9% were found positive by SFDT with lower titers ranging from 1:4 to 1:16. The sensitivity and specificity of the IFATtechnique were 6.6% and 95.9%, respectively. Only 10 cases gave positive agreement both IFAT and SFDT techniques. Seven cases were positive by IFAT, but negative by SFDT. On the other hand, 5 cases were positive by SFDT but, negative by IFAT. The IFAT titers were higher than the SFDT.

DISCUSSION

From the previous literature, the IFAT technique is reliable and agrees with the SFDT. IFAT is usually

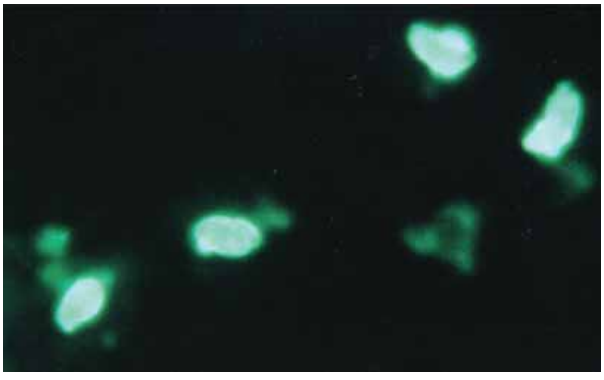

Fig 1a- positive reaction.

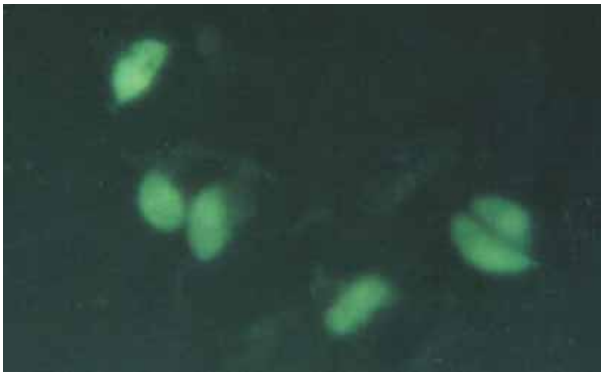

Fig 1b- negative reaction.

Table 1
Comparison of detection for IgG antibody to *T.gondii* in pregnant women with the indirect fluorescent antibody test (IFAT) and the Sabin-Feldman dye test (SFDT).

Test method (IFAT)	Reference method (SFDT)		Total
	No.positive	No. negative	
No.positive	10	17	7
No.negative	5	172	167
Total	15	189	174

accepted as a reference test or a confirmatory test for the results of screening tests in pregnancy in some countries (al-Meshari *et al*, 1989; Azab *et al*, 1993; Alexandre *et al*, 1996). In Thailand, there was no study on pregnant women by IFAT. There was only study for toxoplasmosis in normal persons, lymphoma patients, cancer patients by IFAT, by Tanphaichitra *et al* in 1976. In this study, the seroprevalence of *Toxoplasma* in pregnant women was 7.9% using the dye test and 8.9% using IFAT. IFAT showed 66.6% sensitivity and 95.9% specificity. Therefore, we need to adjust the conditions of the IFAT technique to improve sensitivity and specificity.

The advantage of the IFAT method is that it is safer and simpler to perform than the SFDT, because it does not require living organisms. Killed tachyzoites are smeared on microscopic slides that can be kept at -20˚C for several months before use and could be distributed to laboratories that cannot offer SFDT.

Even though our IFAT had low sensitivity, it yielded 95.9% specificity. This test should be used with caution, especially in immunocompromized hosts, because seropositive individuals need to be identified, to take care of their immune status. However, this test could be good enough to identify seronegative pregnant women. Then, health education concerning disease prevention during pregnancy would be provided.

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