DIFFERENCE OF *TOXOPLASMA GONDII* ANTIBODIES BETWEEN THAI AND AUSTRIAN PREGNANT WOMEN

Yaowalark Sukthana

Department of Protozoology Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand

Abstract. *Toxoplasma gondii* IgG and IgM antibodies of Thai and Austrian pregnant women were studied, in the same laboratory unit, by using the Sabin-Feldman dye test and ISAGA-IgM. In Thai pregnant women, IgG antibody was found in 21.7 %, mostly the IgG titers were low and all were negative for IgM antibody. Conversely in Austrian pregnant women, IgG antibody was found in 30.0% with high titer, and there were 19 (6.3%) cases positive for IgM antibody.

The seropositivity of *T. gondii* IgG antibody in Austrian pregnant women was significantly higher than in Thai ($p = 0.02$) and the titers were much higher. Two possibilities are postulated to explain the data: it may be because Thai women were infected at a younger age than Austrian women, so they were in the chronic infection stage corresponding with their negativity of IgM antibody or it may be due to the difference of strain virulence of *T. gondii* from the different parts of the world.

INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by the protozoan parasite, *Toxoplasma gondii* of which the cat is the only definitive host. It is widely prevalent in man and many species of animals and birds. *T.gondii* is transmitted by three major modes: congenitally, consumption of undercooked or raw infected meat, ingestion of contaminated food and water by oocysts which are shed into environment by cats.

Pregnant women are the population at risk for this disease. This leads the congenital toxoplasmosis, resulting in severe problems such as abortion, congenital anomalies, hydrocephalus, chorioretinitis, mental retardation and hepatosplenomegaly, or symptoms may not appear until adolescence, especially retinitis, which always results in blindness. This is a major problem resulting in a financial burden on families and governments. The infection rate of congenital toxoplasmosis is highest during the third trimester, however, the disease becomes more severe during first trimester infection (Carter and Frank, 1986). The early recognition of maternal infection and treatment with spiramycin or pyrimethamine-sulphadiazine will reduce the parasitic colonization of the placenta and prevent infection in the fetus. If fetal infection has already occurred, maternal treatment modifies the fetal disease. Therapy during the first year of life improves the prognosis (Chatterton, 1992).

The non-specific nature of the signs and symptoms of toxoplasmosis does not permit reliable diagnosis based on clinical findings. Serology is often the investigation of choice. A wide range of antibody detection methods are available. In pregnant women IgG, IgM, IgA antibody detection and avidity test are the recommended methods (Holliman, 1996).

Sabin and Feldman (1948) developed the first reliable serological assay, the dye test, in 1948 and this still remains a definitive test (Holliman, 1996; Chatterton, 1992). But the disadvantages of the dye test are the difficulty of maintaining the inoculated mice and the risk that laboratory personnel may be infected accidentally.

Immunosorbent agglutination assay (ISAGA-IgM) is the method for the detection of specific *Toxoplasma* IgM antibody. It was found to be sensitive and specific technic (Gorgievski *et al*, 1996; Patel and Holliman, 1994; Bitkowska *et al*, 1996; Fricker-Hidalgo *et al*, 1996). But the positive predictive value is limited by the long persistence of IgM after acute infection (Gorgievski *et al*, 1996; Patel *et al*, 1993). Other tests such as an avidity test should be added to differentiate between current and past infection in questionable cases (Chatterton, 1992).

More sensitive and specific diagnostic tools are available in the developed countries. Some countries, Austria and France, have routine ante-natal screening programs for the early detection of toxoplasmosis in pregnant women (Aspoch and Pollak, 1992; Dubey, 1991). Due to the efficient screening programs and treatment of primary toxoplasmosis cases, the congenital infection and complication rate has been reduced in such countries (Aspoch and Pollak, 1992). However, developing countries still

need to establish diagnostic methods and regular screening programs to cope with present problem. Therefore a comparative study of *T.gondii* antibodies in Thai and Austrian pregnant women was designed using the same methods in the same laboratory unit.

MATERIALS AND METHODS

Study groups

This was a cross sectional study. Blood samples from 300 Thai pregnant women were collected consecutively from ANC clinic of Samut Sakhon general hospital. The information regarding age, gestational age, have been recorded. The tested specimens were kept in -4°C and carried to Austria. To make the comparison between Thai and Austrian women, 300 sera from Austrian pregnant women were studied in parallel.

Serological methods

All of serum samples (600) were assayed using the Sabin-Feldman dye test for detection of IgG antibody. Dye positive IgG sera were tested for IgM antibody by using a commercial diagnostic kit (Toxo-ISAGA, BioMerieux). Both methods were performed in the Toxoplasmosis Laboratory Unit, Department of Neonatology and Intensive Care, University Children's Hospital, Vienna, Austria.

The percentage seropositivity of *T.gondii* antibodies was calculated. Then the comparison of background data, percentage of IgG antibody and IgG titer of the two groups were analyzed using Student's *t*-test and chi-square for continuous and categorical variables, respectively.

RESULTS

As indicated in Table 1 there was no significant difference of background data between Thai and Austrian groups regarding age and gestation age. The samples of Thai and Austrian women used were 14-41 years and 18-44 years, respectively. However, most of the study subjects were between 21-30 years old in both groups. More than half of these women were in second trimester pregnancy (55.7% of Thai and 51.3% of Austrian, p=0.06).

There was a statistically significant difference of *T.gondii* IgG antibody between Thai and Austrian pregnant women (21.7% *vs* 30.0%, p=0.02). Table 2 shows the IgG titer of these two groups. In Thai pregnant women, the IgG titers were low, mostly (87.9%) the titers were 1:4 and there were not any higher titer than 1:64. In Austrian women, the majority of Ig G titers (27.8%) were 1:256 and some of them (2.2%) were as high as 1:65,536.

Concerning the IgM antibody, 6.3% (19/300) of Austrian women were positive for IgM, but no Thai women were positive for IgM.

DISCUSSION

Toxoplasma gondii antibody among Thai pregnant women has been reported to vary from 0.8-15.8% previously (Malewong, 1989; Thaiyooth, 1991; Daenesakaew *et al*, 1992; Taechowian *et al*, 1997). This was a low prevalence when compared to 25-80% of pregnant women from Western countries (Holliman, 1996; Chatterton, 1992). In this study the same methods were used in the same laboratory unit, in order to avoid any differrence of laboratory techniques. It was found that even though the IgG seropositivity of Thais was higher than in the past, it was still significantly lower than in Austrian pregnant women (p=0.02). Cats in Thailand are not fed with canned food, which is mostly done in Austria, and they also have more chance to contact with rats or birds. Thailand is located in a tropical area which has the appropriate temperature for oocysts. Many Thai recipes use raw or undercooked meat. Therefore, there are many factors to provoke the transmission of toxoplasmosis in Thailand, yet the prevalence is quite low. The other striking finding was that IgG titers of Austrian women were much higher than in Thais and IgM antibody was found to be positive in Austrian women only. Two possibilities are postulated: Firstly the difference of strain virulence of *T. gondii* itself may play an important role. Secondly, it may be due to infection occurring at a younger age in Thai compared to Austrian women, so they were in the chronic stage of infection. The studies of prevalence of *T. gondii* antibody in the healthy Thai population and the isolation of local *T. gondii* strains are an ongoing process which may answer these postulations.

The higher titer of IgG antibody and positivity of IgM among Austrian women indicated an acute stage of infection during pregnancy, so maternal treatment and ante-natal diagnosis were performed. Children born to positive mothers will be followed up from birth up to one year of age by serial serological studies, with observation of growth and development, and retinal examination by ophthal-

Table 1

Background information of Thai and Austrian pregnant women.

Variabe	No. of Thai pregnant women(%) n=300	No.of Austrian pregnant women(%) n=300
Age		
<20	95 (31.7)	15 (5.0)
21-30	167 (55.7)	187 (62.3)
31-40	30 (10.0)	97 (32.3)
>40	8 (2.7)	1 (0.3)
	p = 1.00	
Gestation age		
1st trimester	77 (25.7)	64 (21.3)
2nd trimester	167 (55.7)	154 (51.3)
3rd trimester	56 (18.7)	82 (27.3)
	p = 0.06	

Table 2

Titer of *Toxoplasma gondii* IgG antibody in Thai and Austrian women.

T.gondii Ig G titer	No. of Thai pregnant women (%) n=66	No. of Austrian pregnant women (%) n=80
1:4	58 (87.9)	6 (6.7)
1:16	7 (10.6)	16 (17.8)
1:64	1 (1.5)	16 (17.8)
1:256	-	25 (27.8)
1:1,024	-	12 (13.3)
1:4,096	-	6 (6.7)
1:16,384	-	7 (7.8)
1:65,536	-	2 (2.2)

mologists. Treatment will be given to children who have clinical symptoms and signs of congenital toxoplasmosis.

Among Thai pregnant women there were not any positive for IgM, consistent with very low titer of IgG antibody which indicated chronic rather than acute infection, thus no further management is needed in this group. Conversely, among the women who were negative to *T. gondii* antibody, serological studies in second and third trimesters should be repeated. The effective strategies of health education about disease transmission must be promoted in this group to prevent primary infection during pregnancy. The studies of primary infection in pregnant women and congenital transmission rate need to be established in this country to cope with the potentially emerging problem.

ACKNOWLEDGEMENTS

The author would like to thanks staff in Toxoplasmosis Laboratory Unit, AKH, University Children's Hospital, Vienna, Austria for their helping in laboratory technique. I am also grateful to staff and nurses in Samut Sakhon Hospital for providing tested samples and collecting data of Thai pregnant women. This work was supported by grant from Austria-Asia Uninet and Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

REFERENCES

Aspoch H, Pollak A. Prevention of prenatal toxoplasmosis by serological screening of pregnant women in Austria. *Scand J Infect Dis* 1992; 84 (suppl): 32-7.

Bitkowska E, Walochowa M, Dzbenski TH. Detection of specific toxoplasma class A antibodies: a comparison of diagnostic usefulness of commercially available diagnostic kits. *Med Dosw Mikrobiol* 1996; 48: 197-200.

Carter AO, Frank JW. Congenital toxoplasmosis: epidemiological features and control. *Can Med Assoc J* 1986; 135: 618-23.

Chatterton JM. Pregnancy. In : Ho-Yen DO, Joss AW, eds. Human Toxoplasmosis. Oxford : Oxford University Press, 1992; 154-83.

Daenesakaew W, Maleewong W, Leevisudpaibul V. Seroprevalence of *Toxoplasma gondii* in pregnant women in Ubon Radhathani. *J Med Assoc Thai* 1992; 75: 609-10.

Dubey JP. Toxoplasmosis-An overview. *Southeast Asian J Trop Med Public Health* 1991; 22 (suppl.): 88-92.

Fricker-Hidalgo H, Pelloux H, Bost M, Goullier-Fleuret A. Congenital toxoplasmosis: contribution of postnatal biological follow-up. *Presse Med* 1996; 25: 1868-72.

Gorgievski HM, Germann D, Matter L. Diagnostic implications of kinetics of immunoglobin M and A antibody responses to *Toxoplasma gondii*. *J Clin Microbiol* 1996; 34: 1506-11.

Holliman RE. Toxoplasmosis. In : Cook GC, ed. Manson' s Tropical Diseases. London: WB Saunders 1996; 1246-54.

Maleewong W. Prevalence of Toxoplasmosis antibody in blood donors and pregnant women in Khon Khaen province. *J Med Assoc Thai* 1989; 72: 256-9.

Patel B, Holliman RE. Antibodies to *Toxoplasma gondii* in eluates from filter paper blood specimens. *Br J Biomed Sci* 1994;51:104-8.

Patel B, Young Y, Tanner RP, Johnson J, Holliman RE. Immunoglobulin-A detection and the investigation of clinical toxoplasmosis. *J Med Microbiol* 1993; 38: 286-92.

Sabin AB, Feldman HA. Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoan parasite. *Science* 1948; 108: 660-3.

Taechowisan T, Sutthent R, Louisirirotchananukul S, Puthavathana P, Wasi C. Immune status in congenital infections by TORCH agents in pregnant Thais. *Asia Pac J Allerg Immunol* 1997; 15: 93-7.

Thaiyooth C. Pattern of antobodies in Toxoplasmosis of pregnant women and their children in Thailand. *Southeast Asian J Trop Med Public Health* 1991; 22 (suppl): 107-10.