

# PREVALENCE OF CONGENITAL CYTOMEGALOVIRUS AND *TOXOPLASMA* ANTIBODIES IN THAILAND

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**Abstract.** Prevalence of CMV and toxoplasmosis antibodies were determined among normal newborn infants, suspected congenital neonates and pregnant women. Seropositive rates of CMV and toxoplasmosis were similar. Most of the pregnant women had CMV antibodies. The in-house ELISA for detection of *Toxoplasma* antibodies was developed and compared with the commercial kit with sensitivity and specificity of 90.47% and 96.74% respectively.

## INTRODUCTION

It has been well established that congenital infection can be caused by various kinds of microorganisms including cytomegalovirus (CMV) and *Toxoplasma gondii*. These organisms can produce similar severe symptoms such as abortion, congenital anomalies, chorioretinitis, mental retardation, hepato-splenomegaly and sometimes with no symptom. The frequencies of these infections are varied in different countries. In Thailand, the prevalence of CMV and toxoplasmosis had been reported 10.2% and 1% respectively in 1980 (Tantivanich *et al*, 1980). Seropositive rates of CMV with higher percentages were also reported in various occupations (Tantivanich *et al*, 1980, 1981, 1982, 1999; Tantivanich and Balachandra, 1986). *T. gondii* antibodies were reported among pregnant women and newborn infants with 2.5% in 1979, 2.8% in 1984, and 13.2% in 1998 from pregnant women and 7.18% to 13.14% from the cord blood (Nabnien, 1979; Morakote 1984; Chintana, 1998). These results indicated that both CMV and *T. gondii* are increasingly important problems among the Thai population. Transmission of CMV usually occurs via sexual contact and infection in pregnant women can lead to congenital infection. For toxoplasmosis, transmission may occur by congenitally, consumption of undercooked infected meat and ingestion of oocyst shed by cats into the environment. While

detection of CMV antibodies was established in our laboratory in 1980 (Tantivanich *et al*, 1980) detection of *T. gondii* still relies on a commercial kit. Therefore, it is interesting to establish an in-house ELISA for detection of *T. gondii* antibodies by comparing with the commercial kits. The prevalence rates of CMV and *T. gondii* antibodies has also been determined and compared between the suspected congenital neonates and the normal newborn infants, as well as among pregnant women.

## MATERIALS AND METHODS

Sera were collected during February 1999 to November 2000 from 3 groups of the population. The first group comprised of 105 suspected congenital neonates from Phramongkutklao Hospital. The second group comprised of 95 normal newborn infants from the Queen Sirikit National Institute of Child Health. The third group comprised of 200 pregnant women who attended the Out Patient Department at the Department of Obstetrics and Gynecology, Rajvithi Hospital, Bangkok.

The CMV antigen was prepared in our laboratory as described previously (Tantivanich *et al*, 1999). The dilution of the antigen of 1:2,000 was used to coat plates.

*T. gondii* antigens were prepared in our laboratory by infecting a confluent monolayer

of HEp-2 cells with *T. gondii* tachyzoites. After 5 days with 3<sup>+</sup> or 4<sup>+</sup> cytopathic effect present, the infected cells were disrupted by glass beads and centrifuged at 2,500 rpm for 10 minutes. The supernatant was sonicated at 10KC/second for 3 minutes, three times, by using an ultrasonic liquid processor (Heat Systems Inc, USA) then centrifuged at 2,500 rpm for 10 minutes and the supernatant kept as the antigen. A dilution of the antigen of 1:800 was used to coat plates.

The enzyme-linked immunosorbent assays (ELISA) for measuring CMV and *T. gondii* antibodies were performed as described by Tantivanich *et al* (1980). For detecting IgM antibody, normal human IgG antibody was used to block the non-specific reaction by incubating the IgG antibody with the test sera one hour before performing the test. ELISA IgG and IgM antibodies were determined using peroxidase-conjugated rabbit anti-human IgG specific for gamma chain and peroxidase-conjugated rabbit anti-human IgM specific for Mu-chains (Dako). The dilutions of the conjugate were 1:2,000 as recommended by the manufacturer. ELISA titers of IgG and IgM antibodies were considered positive if the titers exceeded 1:80 and 1:320 for CMV and toxoplasmosis respectively.

The commercial kit for detection of antibody to *T. gondii* was purchased from Sanofi Diagnostics Pasteur, France. One hundred sera from pregnant women, suspected congenital

neonates and normal newborn infants were randomly selected to compare the in-house ELISA with the commercial kits.

The IgG and IgM antibody titers comparison between suspected congenital neonates and normal newborn infants were analyzed by chi-square and Fisher exact tests.

RESULTS

The prevalence of CMV and *T. gondii* antibodies of suspected congenital neonates and normal newborn infants is shown in Table 1. The seropositive rates of CMV antibodies among these two groups of infants were similar with no significant difference (p>0.05). Only 1 out of 105 (0.9%) suspected congenital neonates had IgM antibody. For *T. gondii* antibodies, the seropositive rates of IgG antibody of the normal newborn infants was significantly higher than the suspected congenital neonates (p<0.05). Mixed infections of CMV and *T. gondii* were also detected. Similar percentages of IgG antibody were found in both groups with no IgM antibody.

Comparison between CMV infection and toxoplasmosis found that the toxoplasmosis had higher seropositive rates than CMV among the normal newborn infants with no significant difference (p>0.05), while the suspected congenital neonates had similar percentages with no significant difference (p>0.05).

Table 1  
Prevalence of CMV and *T. gondii* antibodies in suspected congenital neonates and normal newborn infant.

Diseases	Normal newborn infants			Suspected congenital neonates			p-value
	IgG(%)	IgM(%)	IgG+IgM(%)	IgG(%)	IgM(%)	IgG+IgM(%)	
CMV	11 (11.57)	0 (0)	0 (0)	12 (11.42)	1 (0.95)	0 (0)	0.973 <sup>a</sup>
Toxoplasmosis	20 (21.05)	0 (0)	0 (0)	9 (8.57)	0 (0)	0 (0)	0.012 <sup>a</sup>
CMV and toxoplasmosis	3 (3.1)	0 (0)	0 (0)	2 (1.95)	0 (0)	0 (0)	0.670 <sup>b</sup>

<sup>a</sup> Chi-square test: Normal newborn infants vs suspected congenital neonates.

<sup>b</sup> Fisher's exact test: Normal newborn infants vs suspected congenital neonates.

Table 2  
Prevalence of CMV and *T. gondii* antibodies in 200 pregnant women.

Disease	IgG (%)	IgM (%)	IgG+IgM (%)	Negative (%)
CMV	141 (79.7)	2 (1.1)	24 (13.6)	10 (5.6)
Toxoplasmosis	24 (13.6)	3 (1.7)	11 (6.2)	139 (78.5)
CMV and toxoplasmosis	22 (11.0)	0 (0)	1 (0.05)	177 (88.5)

Among the pregnant women, CMV gave higher percentages of IgG and IgM antibodies than toxoplasmosis. Mixed infections of CMV and *T. gondii* were also detected (Table 2).

The in-house ELISA and commercial kit were compared among 100 randomly selected sera. By using the commercial kit as a standard method, it was found that the in-house ELISA had sensitivity and specificity of 90.47% and 96.74% respectively.

## DISCUSSION

The studies of congenital infection by TORCH agents had been reported earlier (Tantivanich *et al*, 1980). CMV was reported as the second rank next to rubella while toxoplasmosis was found to be the least important organism because of its low seropositive rate. At the present time, rubella is not considered to be an important problem anymore because of a good vaccination program. The infection by herpes simplex virus has also decreased possibly because of the epidemiology of HIV which has changed the human behavior. The remaining problems that should be considered now are CMV and toxoplasmosis.

The seropositive rates of CMV antibodies among normal newborn infants and suspected congenital neonates in this study were similar and most of them had IgG antibodies, which indicated the passive transfer antibodies from their mothers. These results were similar to the results of previous study in 1980 (Tantivanich *et al*, 1980). Most of the pregnant women also had IgG antibodies which indicated past infection and this is the reason why most of the newborn infants had IgG antibodies. Unfor-

tunately, we did not have the sera from the mothers and their neonates but the results of this study indicated that CMV is still one of the important microorganisms that cause congenital infection.

Toxoplasmosis antibodies of normal newborn infants in this study were higher than CMV antibodies. Even though the sample sizes were small, this result indicated that toxoplasmosis has now become as important as CMV in causing congenital infection.

The seropositive rates of *Toxoplasma* antibody of the normal newborn infants were higher than the suspected congenital neonates, possibly for the following reasons: 1). The mothers of the normal newborn infants were infected with *T. gondii* more than the mothers of the suspected congenital neonates. 2). The mothers of the suspected congenital neonates already passed the infection to their neonates with undetectable sign of infections (Robert-Gangneux *et al*, 1999).

Since this is a preliminary study, further investigation should be done in groups of mothers and their newborns in order to confirm the infection rates.

From the results of this study it can be concluded that CMV and toxoplasmosis are health problems in Thailand. Health education concerning the transmission of *T.gondii* and CMV are strongly needed in order to prevent the spread of the diseases. Early diagnosis of toxoplasmosis and CMV are also important. A good standard and reliable method, especially in-house ELISA, should be introduced in various laboratories, especially in the rural area, in order to save the budget of the country.

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