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Immune Status in Congenital Infections by TORCH Agents in Pregnant Thais

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SUMMARY **A cross-sectional, sero-epidemiological survey of the prevalence of antibodies to TORCH agents during various stages of gestation revealed an overall rate of 13-15 percent having antibodies to *Toxoplasma gondii*; 85-87 percent, to rubella ; 79-81 percent, to herpes simplex virus (HSV); 100 percent, to cytomegalovirus (CMV); 82-86 percent, to human herpes virus type 6 (HHV-6); 1-2 percent, to hepatitis C virus (HCV). None of human T lymphotropic virus type I (HTLV-I) antibody was detected, and a prevalence of hepatitis B surface antigen (HBsAg) was 6 percent. Although a tendency was noted towards an increase of antibody detection to each TORCH agent as gestation progressed, a statistically significant increase in antibodies titer and specific IgM antibody was found with regard to CMV. These results suggest an increase in CMV infection or reactivation during pregnancy whereas an increase in the other TORCH infections was not obvious.**

The term TORCH is the acronym indicates the role of several pathogens that produce similar abnormalities in the infected fetus and the newborn.[1] Eventhough additional agents have been shown to produce lesions of a similar nature, *Toxoplasma gondii*, rubella, cytomegalovirus (CMV), and herpes simplex virus (HSV) are the ones most frequently investigated,[2] whereas others have simply reminded themselves by the "O" in "TORCH" that there are "other" infectious agents that may cause similar congenital pathology.[3] Hepatitis B virus (HBV), hepatitis C virus (HCV), human T-lymphotropic virus type I (HTLV-I) and human herpes virus type 6 (HHV-6) are newly recognized viruses that could be transmitted vertically.[4-6] The fact that many of the neonatal infections caused by TORCH agents are congenitally acquired has been substantiated in a number of sero-epidemiological studies on pregnant women. The present cross-sectional study was designed to estimate the prevalence of TORCH agents among pregnant women in Bangkok.

MATERIALS AND METHODS

Sera

Serum specimens were collected from pregnant women who attended the antenatal care clinic at Siriraj Hospital from September 1992 to June 1995. They were between 14 and 40 years of age. The serum specimens were sampling in every tenth, 100 each from the groups of first trimester (1-3 months), second trimester (4-6 months) and third trimester (7-9 months) of gestation. For HTLV-I antibody, additional samples of three hundred samples from each trimester were tested. These serum specimens were kept at -35°C until tested.

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Serological methods

Laboratory diagnosis of TORCH infections in the pregnant women was performed by TOXO-ELISA II (Biowhittaker, USA) for *T. gondii* IgG antibody, RUBELISA II (Biowhittaker, USA) for rubella IgG antibody, CYTOMEG-ELISA II (Biowhittaker, USA) for CMV IgG antibody, HERPELISA II (Biowhittaker, USA) for HSV IgG antibody, HTLV-I serodia (Fujirebio, Japan) for total antibody to HTLV-I, HCV-EIA (Abbott, USA) for IgG antibody to HCV, HBsAg-RPHA (Fujirebio, Japan) for HBsAg and indirect immunofluorescent assay (IFA) for HHV-6 (Hashimoto strain, Japan) for IgG antibody to HHV-6. The CMV seropositive sera were further investigated quantitatively by passive particle agglutination test for CMV antibody titer. The positive sera for IgG antibody to CMV, *T. gondii* and rubella were tested for IgM antibody by CMV-STAT M, TOXOSTAT M and RUBESTAT M (Biowhittaker, USA), respectively.

RESULTS

The results of the serological screening against TORCH agents in pregnant women, regardless of their gestational ages are shown in Table 1. *T. gondii* IgG antibodies were detected in 13-15 percent. CMV antibodies were found to be present in 100 percent of these pregnant women. HSV IgG antibodies were found in 79-81 percent. High prevalence (85-87 percent) of rubella IgG antibodies was demonstrated by ELISA, indicative of the immune status of these pregnant women. Using an IFA test, HHV-6 IgG antibodies were found in 82-86 percent. Further, a prevalence of HBsAg was 6 percent, anti-HCV was 1-2 percent and none of HTLV-I antibody was detected.

Quantitative CMV antibody titers by passive agglutination among pregnant women at the various gestation period are shown in Table 2. The results showed that 100 percent seropositive rates were found in all three groups with the GMT of the antibody titers were 1:430, 1:1,002 and 1:1,379 in first, second and third trimesters of gestation, respectively. There was significant difference in GMT of the women in the first and second trimesters of gestation ($p<0.05$) but there was no significant difference in GMT of the women in the second and the third trimesters of gestation.

Table 1. Prevalence of antibodies to TORCH agents in pregnants by gestation ages.

Serological test for	No. Positive/ No. tested (%) at		
	1st Trimester	2nd Trimester	3rd Trimester
Antibodies to			
T. gondii (ELISA)	15/100 (15%)	13/100 (13%)	13/100 (13%)
Rubella (ELISA)	85/100 (85%)	85/100 (85%)	87/100 (87%)
CMV (ELISA & PA)	100/100 (100%)	100/100 (100%)	100/100 (100%)
HSV-2 (ELISA)	79/100 (79%)	81/100 (81%)	80/100 (80%)
HHV-6 (IFA)	82/100 (82%)	85/100 (85%)	86/100 (86%)
HTLV-I (PA)	0/300 (0%)	0/300 (0%)	0/300 (0%)
HCV (ELISA)	1/100 (1%)	1/70 (1.4%)	2/100 (2%)
Antigen to			
HBsAg (RPHA)	6/100 (6%)	6/100 (6%)	9/150 (6%)

Table 2. Distribution of CMV antibody titers in preganant women by gestation ages

Gestation age	$\bar{X}$ (wk.)	No. of pregnants with CMV reciprocal antibody titers									GMT	Total Tested
		64	128	256	512	1,024	2,048	4,069	8,192	16,384		
1st Trimester	11	13	15	15	25	17	7	4	4	-	430	100
2nd Trimester	24	-	5	17	19	21	18	14	5	1	1,002	100
3rd Trimester	35	-	2	15	17	19	19	11	12	5	1,379	100

$\bar{X}$: mean gestation ages.
GMT : geometric mean titer.

Table 3. Specific IgM antibodies to *Toxoplasma gondii*, rubella and CMV in Thai pregnancies

Specific IgM antibodies to	No. positive/ No. tested (%) at		
	1st Trimester	2nd Trimester	3rd Trimester
Toxoplasma gondii	1/15 (6%)	1/13 (7%)	1/13 (7%)
Rubella	1/50 (2%)	1/50 (2%)	2/50 (7%)
Cytomegalovirus	2/50 (4%)	7/50 (14%)	8/50 (16%)

The detections of specific IgM antibodies to *T. gondii*, rubella and CMV were shown in Table 3. *Toxoplasma* IgM antibodies were found in one woman from each of the first, second and third trimesters of pregnancy group. Of the 85% pregnant women in each trimester who were positive for rubella IgG antibodies, rubella specific IgM was demonstrated in 4 women, one each at the first and the second trimesters and 2 at the third trimester. Among all pregnant women who possessed CMV antibodies, the positivity for anti-CMV IgM antibodies during various stages of gestation was different. The positivity rates of anti-CMV IgM antibodies were 4%, 14% and 16% in first, second and third trimesters of gestation, respectively.

DISCUSSION

The results of this sero-epidemiological study indicate a various prevalence of antibodies to each of the agents tested among pregnant women in Bangkok. The present investigation have effectively utilized the ELISA for the detection of IgG antibodies to *T. gondii*. Sero-epidemiologic study of the pregnant women indicated the presence of antibodies due to past *Toxoplasma* infection. The presence of IgM antibodies confirmed the recent infection with *T. gondii* in one

each of the first, second and third trimesters of pregnancy. Studies reported in the literature indicated 17 percent seropositivity to *Toxoplasma* with specific *Toxoplasma* IgM detected in 1 to 2 per 1,500 pregnants in Britain and mainland Europe.[7] Comparison with these findings indicate a much higher incidence of specific *Toxoplasma* IgM.

Rubella, if acquired during the first trimester of pregnancy can damage the developing fetus. The serological testing to accurately determine the immune status in women of child bearing age is of extreme importance. In a previous sero-epidemiological study of rubella in pregnancies, Puthavathana *et al.*,[8] using the haemagglutination inhibition test with kaolin absorption of non-specific inhibitor, found seropositivity of 75% in Thai pregnancies. In the present study, ELISA rubella antibody test system was used. The ELISA shown with maximum sensitivity and which is reliable as well as rapid. This study found a prevalence of 85-87% of rubella antibodies in the pregnant Thai women. The higher seropositive rate to rubella in this study might reflect the higher sensitivity of ELISA than HI or an increase of rubella immunity. There were cases which were positive for rubella IgM during third trimester of pregnancies and one each at the first and second trimesters of gestation. During the past few years, very few cases of rubella were seen. This reason due to the effect of nationwide immunization with live attenuated rubella vaccine during childbearing years for preventing congenital rubella in future generations.

Both HSV type 1 and 2 may cause genital herpes and neonatal disease. These results are similar to a previous study which reported 81% of HSV antibody in Thai pregnancies in 1983, even when the complement-fixation test was used.[8] This high prevalence of antibodies were attributed to mode of transmission and the socioeconomic conditions of the population.

HHV-6 causes examthem subitum as a primary infection in children.[9] Furthermore, it can be supposed from the serological studies that infants are infected with HHV-6 when the titer of maternal antibodies declines.[10] So it is possible that HHV-6 can cause congenital infections. The seropositive rate was 82-86% and there was no difference in the prevalence of antibody against HHV-6 among the pregnant women at difference gestation period. These data agreed with those reported by Balachandra *et al.*,[11] that no significant difference was detected in antibody positive rate and antibody titer during various stages of gestation.

The presence of HBsAg in the maternal serum indicates the presence of HBV infection, and mother-to-infant transmission has been reported.[12] The serological screening for HBsAg was found in 6% of the Thai pregnant women. In a previous study, Kanchanaraksa *et al.*[13] reported 7-11% of the Thai population had persistent HBsAg antigenemia or were carriers of HBV in 1987. HCV is a major problem of post-transfusion hepatitis. This virus can be transmitted vertically.[14] Presence of antibodies to HCV is one of the indicators of HCV infection. When the prevalence of HCV appears to be low, about 1-2%, then congenital infection caused by HCV is suspected. HTLV-I infection has also been reported in children who have not been breast-fed which suggests the possibility of intrauterine and intravaginal infection.[15] All of the women were negative for antibodies to HTLV-I. These results suggest there was no HTLV-I infection in this group of the pregnant Thai women. According to Chiewsilp *et al.*,[16] there was no HTLV-I infection in donated blood or in thalassemic patients in Thais.

Serodia-CMV was used for the screeing for CMV total antibodies. CMV antibodies were detected in 100 percent of these pregnant women. This extremely high prevalence of CMV antibodies is comparable to results reported from various parts of the world which ranged from 40-100%. The prevalence of antibody is low in Europe, Autralia, and parts of North America, whereas it is significantly higher in the developing countries of Africa and Southeast Asia.[17] A previous report showed that 82-83% of pregnant Thais at different gestational ages had CMV CF antibody.[8] In 100% who were seropositive for CMV total antibodies, specific CMV-IgM antibodies were detected in 4% of pregancies in first trimester, 14% of second trimester and 16% of third trimester. These results suggested that there was a difference in the prevalence of specific CMV-IgM antibody among three groups of pregnant women at different gestation periods. The presence of higher levels of specific CMV-IgM in second and third trimesters shows that CMV was either a reactivated virus or was obtained from an exogenous source. This finding corresponds to the studies by Stagno *et al.*,[18] that the specific

CMV-IgM increases progressively during pregnancy. In addition, antibody to CMV was quantitated among first, second and third trimesters of gestation. The geometric mean titer (GMT) of the antibody titers were 1:430, 1:1,002 and and 1:1,379, respectively. These results suggested that CMV antibody levels were significantly lower in the first trimester group than in the second and third trimester groups ($p<0.05$). It has been reported that the reactivation of CMV can be obseved during pregnancy, and the virus was readily isolated from cervical and urinary tracts. However, the increase of antibody titer was found in a few cases during early pregnancy.[19]

These studies, showing a rise in antibody titer suggesting new or recurrent infections during pregnancy, should add weight to this concern. It should be pointed out that these rises could have been shown even without excluding multiparous subjects from the study. Multiparity could mask such rises simply because of the greater chance of having either an infection and/or a higher baseline level during a previous pregnancy, thus making any rise less obvious. By the same token, a rise among primigravidae should be even more easily shown, particularly when the baseline prevalence and titer level of nulligravidae are included. Possibly for this reason, the apparent rise in the levels of the other agents, *ie. T. gondii*, rubella, HSV, HHV-6, HBV, HCV, and HTLV-I, in the present study was not substantiated.

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