Sero-epidemiology of TORCH Agents Among 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 14 per cent having antibodies to *Toxoplasma gondii;* 75 per cent, to rubella virus; 82 per cent, to cytomegalovirus (CMV); and 81 per cent, to herpes simplex virus (HSV). Although a tendency was noted towards an increase of antibody titre to each TORCH agent as gestation progressed, a statistically significant increase in titre was found only with regard to CMV. These results suggest an increase in CMV infection during pregnancy whereas an increase in toxoplasma, rubella and herpes simplex virus infections was not so obvious.**

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TORCH agents *(referring to toxoplasma, rubella, cytomegalovirus and herpes simplex virus)* are the most common cause of infection in newborns.[1,2] Infection, either primary or recurrent, that occurs during pregnancy can affect either the fetus *in utero* or the child at the time of delivery.

Pregnant women are said to be more susceptible to a variety of infections than the population at large.[3,4] Both a decrease in cell-mediated immunity to and an increase in the virus isolation rate of CMV in late pregnancy have been reported.[5,6] 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 and newborns thus affected. These studies not only revealed the prevalence of infection but also suggested the mode of transmission to offspring. Furthermore, a significant rise in specific antibody titres as gestation progresses can indirectly suggest an increase in the prevalence of infection with regard to specific stages of gestation, whereas determination of immunoglobulin classes in the cord blood or sera of the newborn at birth helps in discriminating between the transplacental transmission of the TORCH agents versus their acquisition at delivery. However, longitudinal serological studies can be influenced by outbreaks occurring during the study period. The present cross-sectional study was designed to minimize this influence in an attempt to estimate the prevalence and changes in the titre of antibodies to TORCH agents among pregnant women in Bangkok.

MATERIALS AND METHODS

Sera. Sera employed were obtained from pregnant women during various stages of gestation. These women were visiting the antenatal-care clinic at Siriraj Hospital during the period 1979 to 1981. They were between 18 and 35 years of age. Their sera were kept at -20°C until tested.

Antigen. CMV and HSV–1 antigens for use in the complement fixation tests were provided partly by the World Health Organization; others were purchased from the Behring Institute, Germany. Toxoplasma antigens for use in the passive haemagglutination tests (Cellognost) were purchased from the latter Institute. Rubella haemagglutinating antigens came from Flow Laboratories, U.S.A.

Passive haemagglutination test for toxoplasma. The test was done in microtitre U-plates using all the reagents supplied in the Cellognost kits. The sera were diluted serially from 1:8. Antibody titre was read at the highest serum dilution level that gave rise to at least 25 per cent haemagglutination of toxoplasma-sensitized red blood cells of sheep and did not show nonspecific agglutination with unsensitized red cells.

Haemagglutination inhibition test for rubella.[7] The test was done in microtitre V-plates in order

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to detect the rubella antibody. To get rid of any nonspecific inhibitor or nonspecific agglutinator in the sera, each serum sample was treated with kaolin, 50 per cent red blood cells of pigeon and then inactivated at 56°C for 30 minutes. HEPES saline albumin gelatin of pH 6.2 was used as a working buffer in the test. The treated sera were diluted two-fold serially from 1:10 to 1:320, and rubella antigens were used at concentrations of 4-8 haemagglutination units.

The antibody titre was read at the highest serum dilution level that completely inhibited haemagglutination, and at the point when the serum control well did not show nonspecific agglutination activity.

Complement-fixation test for CMV and HSV.[7] The test was performed in microtitre U-plates to assay antibody titre to CMV and HSV. Guinea pig sera were used as the source of complement at a working concentration of 1.5-2.0 haemolytic units. Veronal buffered saline of pH 7.2 was used. The sera were inactivated at 56°C for 30 minutes, and diluted two-fold serially from 1:8.

The CF titre was read at the highest serum dilution level that gave rise to less than 50 per cent haemolysis of the sensitized red blood cells. Sera showing anti-complementary activity or the presence of antibodies to the control antigens were excluded.

RESULTS

Antibodies to toxoplasma, rubella, CMV and HSV. Sera from all the pregnant women, regardless of their gestational stages, were screened for antibodies to toxoplasma at a dilution of 1:8 using the passive haemagglutination test; to rubella at a dilution of 1:10 using the haemagglutination inhibition test; and to CMV and HSV at a dilution of 1:8 using the complement-fixation test. The results in Table 1 show that 14.22 per cent of the women tested had antibodies to toxoplasma; 75.4 per cent, to rubella; 82.3 per cent, to CMV; and 81.5 per cent, to HSV.

Antibody titres to toxoplasma, rubella, CMV and HSV in women at different gestational stages. Sera were collected from three groups of pregnant women, i.e., during their first, second and third trimester, in order to assess possible changes in antibody titres in relation to gestational stages. The distribution of the number of sera at various levels of antibody titre to toxoplasma, rubella, CMV and HSV for each of the three groups is shown in Table 2 through 5.

Table 1 Antibodies to TORCH agents in pregnant women

Agents	No. tested	No. positive
Toxoplasma gondii	232	33 (14.22%)
Rubella	203	153 (75.36%)
Cytomegalovirus	277	228 (82.31%)
Herpes simplex virus	287	234 (81.53%)

The number of women with higher antibody titre levels seemed to increase in the later stages of ges-

Table 2 Toxoplasma antibody titres among pregnant women*

Trimester	No. of sera samples at reciprocal antibody titres of						No. of positive samples
	< 8	8	16	32	64	128	
First	29	4	1	0	0	0	5/34 (14.7%)
Second	49	1	4	1	0	1	7/56 (12.5%)
Third	53	8	0	3	1	0	12/65 (18.46%)
Total	**131**	**13**	**5**	**4**	**1**	**1**	**24/155 (15.48%)**

*There was no statistical difference in antibody titres according to the trimester of gestation ($p > 0.05$: X^2 –test)

Table 3 Rubella antibody titres among pregnant women*

Trimester	No. of sera samples at reciprocal antibody titres of							No. of positive samples
	< 10	10	20	40	80	160	320	
First	12	2	11	9	8	3	0	33/45 (73.33%)
Second	19	6	7	14	15	7	4	53/72 (73.61%)
Third	19	7	7	20	12	13	8	67/86 (77.9%)
Total	**50**	**15**	**25**	**43**	**35**	**23**	**12**	**153/203 (75.36%)**

*There was no statistical difference in antibody titres according to trimester of gestation ($p > 0.05$: X^2 –test)

tation thus suggesting an increase in the prevalence of infection during pregnancy. However, an X^2 -test for discerning the difference in specific antibody titres showed that only CMV antibody titre levels changed significantly ($p<0.01$) according to gestational stage (Table 4). As may be observed in Table 2, 3 and 5, there was no obvious increase in antibody titres to toxoplasma, rubella and HSV ($p>0.05$).

DISCUSSION

The results of this preliminary sero-epidemiological study indicate a high prevalence of antibodies to each of the TORCH agents tested among pregnant women in Bangkok. However, whether the high prevalence for each of the agents implies an increase rate of infection during pregnancy itself remains to be further investigated. For this purpose, a determination of the baseline prevalence of antibodies among nulligravidae is a necessary yard-stick for assessing a possible increase in the prevalence of or a rise in specific antibody titre during the various stages of pregnancy. Some effect on multigravidae should be expected with regard to the prevalence of antibodies and antibody titre. Furthermore, the frequency of antibody detection, particularly of antibodies of the IgM class found in the cord blood or in the sera of the newborn thus indicating intra-uterine infection, could also indicate the presence of an active infection during pregnancy.

Unfortunately, such data on each of the agents (with perhaps the exception of rubella and CMV) are scarce for Thailand. Our earlier studies on rubella revealed that, subsequent to an outbreak of rubella (September 1967 to May 1968), the prevalence in Bangkok of seropositive women (mostly of childbearing age) was approximately 52 per cent[8] and the prevalence among pregnant women subsequent to another outbreak (September 1978 to April 1979) was approximately 77 per cent.[9] These findings are substantiated by the present report carried out over the period described in Table 1. The prevalence of seropositivity to CMV among various groups of subjects more than nine years of age ranged from 79 to 86 per cent; in cord blood the prevalence and antibody titre levels were reportedly higher than among adults.[10] The high prevalence of seropositivity for rubella and CMV as previously reported and confirmed in the present study and for HSV as reported here indicate that these agents are widespread in Thailand and that infection of newborn children could be a major problem. This is particularly true for CMV, if it can be substantiated that: (1) recurrent rather than primary maternal infection is associated more often with perinatal infection as suggested by others;[11] and (2) maternal antibodies, while reducing

Table 4 CMV antibody titres among pregnant women*

Trimester	No. of sera samples at reciprocal antibody titres of					No. of positive samples
	<8	8	16	32	64	
First	9	11	9	4	2	26/35 (74.28%)
Second	10	19	22	3	0	44/54 (81.48%)
Third	6	20	17	19	4	60/66 (90.9%)
Total	**25**	**50**	**48**	**26**	**6**	**130/155 (83.87%)**

***The difference in antibody titres according to trimester of gestation was statistically significant. ($p<0.01$: X^2 – test)**

Table 5 HSV antibody titres among pregnant women*

Trimester	No. of sera samples at reciprocal antibody titres of					No. of positive samples
	<8	8	16	32	64	
First	10	8	15	3	0	26/36 (72.22%)
Second	10	11	23	9	2	45/55 (81.81%)
Third	11	11	28	12	4	55/66 (83.33%)
Total	**31**	**30**	**66**	**24**	**6**	**126/157 (80.25%)**

***There was no statistical difference in antibody titres according to gestational stages ($p>0.05$: X^2 – test)**

the severity of infection, are not effective in preventing perinatal infection.[12] The results of the present report (Table 4), showing a rise in prevalence and antibody titre level thus suggesting new or recurrent infection 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 titre level of nulligravidae are included. Possibly for this reason, the apparent rise in the levels of the other agents, i.e., toxoplasma, rubella and HSV, in the present study was not substantiated by statistical analyses. Current efforts to establish both a baseline for nulligravidous controls and inclusive studies on primigravidae should confirm a rise in cases of CMV and may uncover a rise in cases of infection by the other agents in the TORCH group. To further substantiate that there is an increase in TORCH infection during pregnancy, IgM antibodies in cord sera should be investigated even after an apparently healthy birth.

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