

Seroprevalence of TORCH-S Infections among Pregnant Woman: A Study from Vellore District (South India)

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Abstract

Introduction: TORCH-S agents include *Toxoplasma gondii*, Rubella virus, *Cytomegalovirus*, Herpes simplex virus (HSV) (1 and 2), and *Treponema pallidum* (syphilis) which are transmissible in utero at various stages of gestation. **Description of the Hypothesis Tested:** TORCH-S agents are known to cause adverse fetal outcomes and pregnancy loss. **The Approach Used:** Pregnant women attending a multispecialty hospital for regular antenatal care and high-risk pregnant women with a bad obstetric outcome from a rural area of Vellore District were recruited. A total of 180 pregnant women recruited from two centers were used. Pregnant women were evaluated for their serological status (IgM and IgG) against TORCH-S agents using commercial enzyme-linked immunosorbent assay kits available for respective pathogens. **Results:** Among the samples ($n = 180$) collected, IgM antibodies were positive in 3 (1.66%) for *Toxoplasma gondii* and 1 (0.55%) for HSV1. IgG antibodies were positive in 14 (7.77%) women for *T. gondii*, 152 (84.44%) for Rubella virus, 110 (61.11%) for CMV, 125 (69.44%) for the HSV-1 (16.66%), 30 were positive for HSV-2, and 5 (2.77%) women were positive for *Treponema pallidum*. In the 17–25-year age group, the number of IgG positives for *T. gondii* and HSV-2 were lower compared to other pathogens. **Conclusions:** The study reports a high prevalence of IgG to TORCH-S agents in pregnant women indicating a high risk among these populations. Routine screening for TORCH-S agents among antenatal women is warranted as timely diagnosis, and proper intervention could help initiate appropriate management. Information of these infections could help the clinicians for appropriate counseling on the potential for adverse fetal outcomes and preventive measures to the mothers.

Keywords: Bad obstetric history, enzyme-linked immunosorbent assay, high-risk pregnancy, IgG, IgM, TORCH-S infections

INTRODUCTION

TORCH-S is a medical acronym for a set of perinatal infections with known adverse impact on fetal development and pregnancy outcome. This includes infections with *Toxoplasma gondii*, Rubella virus, *Cytomegalovirus*, Herpes simplex virus (HSV) (1 and 2), and *T. pallidum*.

Toxoplasmosis is a major cause of congenitally acquired infection and it has been documented as the main reason of bad obstetric history (BOH), primarily leads to fetal death and morbidity of the newborns. Toxoplasmosis among pregnant women could lead to spontaneous abortions, stillbirths, intrauterine growth retardation, preterm deliveries, or fetal damage.^[1] Among pregnant women during the 1st week of pregnancy, rubella virus infection causes devastating problems, leading to hearing loss, cataracts, congenital heart defects, neurological problems, hepatomegaly, and

splenomegaly and are collectively known as congenital rubella syndrome (CRS).^[2] About 40%–50% of *Cytomegalovirus* infected pregnant women could transmit the virus to the fetus. Among pregnant women, the *Cytomegalovirus* transmission from mother to fetus could occur even if the mother was infected earlier before pregnancy.

Among pregnant women, genital herpes has been documented with spontaneous abortions, intrauterine growth retardation,

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preterm labor, congenital, and neonatal herpes infections.^[3] *T. pallidum* can be transmitted to the infant by an infected pregnant mother or at birth through contact with maternal lesions.^[4] If the infection is untreated, it may lead to complications such as early fetal loss, preterm birth, and low birth weight.^[5]

The diagnosis of TORCH-S infections mainly depends on serological testing as these maternal infections are initially asymptomatic and the clinical diagnoses are unpredictable. The presence of IgM antibodies indicates a recent infection and the presence of IgG antibodies indicates past infection. The detection of the IgM and IgG antibody is the best approach for the identification of TORCH-S infections. The present study was to evaluate the serological status of pregnant women against TORCH-S agents by detection of IgM and IgG using enzyme-linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Pregnant women were recruited prospectively for the cross-sectional study using a random sampling method. A total of 140 serum samples from pregnant women attending Department of Obstetrics and Gynaecology at Sri Narayani Hospital and Research Centre (SNHRC), Sripuram, Vellore, a multispecialty hospital mainly serving rural and periurban populations of Vellore district. Forty serum samples were collected from pregnant women attending upgraded Primary Health Center (PHC), Ussoor, in the rural area of Vellore District. The pregnant women ($n = 140$) recruited from SNHRC were otherwise asymptomatic who came for routine checkups. The women ($n = 40$) identified as a high-risk group were recruited from the PHC during the special camp organized for women with “high-risk” pregnancy. Permission for collecting the samples from the PHC was obtained through the Department of Public Health, Vellore (District office) and Directorate of Public and Preventive Medicine, Chennai (Head office). A clinical questionnaire and written consent were obtained from each pregnant woman. This study was approved for ethical clearance by SNHRC Ethical Committee (No: IEC/IRB No: 21/04/06/11, dated: 04/06/2011).

Inclusion criteria

Pregnant women attending antenatal clinic at SNHRC and high-risk pregnant women camp at Ussoor PHC who gave consent to participate in the study were included in the study.

Exclusion criteria

Women who did not give informed consent to participate and had conditions irrelevant to the proposed study clinical groups were excluded from the study.

This study was conducted from March 2017 to February 2019. Demographic details of the pregnant women attending SNHRC ($n = 180$) including maternal age, domiciliary status, occupation, and educational level were collected. Clinical information including hemoglobin level and history of abnormal pregnancies such as two or more consecutive spontaneous abortions, intrauterine fetal death, congenital

malformation, and stillbirth were collected using a structured standard questionnaire. Information on pregnant women attending upgraded PHC ($n = 40$) was very limited. Two milliliter of venous blood was collected into BD Vacutainer® serum tubes. Serum was separated and stored immediately at -80°C deep freezer until tested.

TORCH-S IgG and IgM enzyme-linked immunosorbent assay

TORCH-S IgG and IgM antibodies were detected from the serum by commercially available ELISA test-kit (Calbiotech Inc., Canada). The test was performed according to the manufacturer’s instructions. Briefly, the serum was diluted at 1:21 with dilution buffer for all the TORCH-S ELISA except for *T. pallidum* IgM assay, the dilution was 1:51. The negative control, positive control, and calibrator control were added into the appropriate wells. After loading the sample into a 96-well plate, the plate was covered with an adhesive and incubated for 20 min at room temperature, for *T. pallidum* IgM assay alone, it was incubated for 45 min at 37°C . After washing thrice with 300 μl of 1X wash buffer, 100 μL of the conjugate solution was dispensed in each well. Then, the plate was covered again and incubated for 20 min at room temperature and 45 min at 37°C for *T. pallidum* IgM assay. After washing thrice with 300 μl of 1X wash buffer, 100 μL of TMB substrate was dispensed into each well, avoiding the formation of bubbles and incubated for 10 min at room temperature in dark and for *T. pallidum*, the plate was incubated in dark for 15 min at room temperature. The reaction was stopped using 100 μl of stop solution.

TORCH-S antibody index was calculated by dividing the value of each sample by calibrator values. ELISA results were recorded using a microplate reader (Mindray MR-96A, Germany), as a measure of optical densities (OD) of the reaction intensity of TORCH-S IgG and IgM antibodies at a filter wavelength of 450 nm. Cut-off points and antibody index calculations were done according to manufacturers’ recommendation to categorize seropositive (antibody index >1.1), borderline positive (antibody index 0.9–1.1), and seronegative (antibody index <0.9) samples. In this study, all serum samples with intensity of antibody index 0.9–1.1 (borderline positives) were not considered.

RESULTS

In our study, out of 180 random samples tested by IgM assay for the TORCH-S agents, three samples (1.66%) were positive for *T. gondii* and one sample (0.55%) was positive for HSV-1. Rubella virus, CMV, HSV-2, and *T. pallidum* were negative among the sample tested.

The study population included 180 pregnant women in the age group of 17–41 years. The median age of the study population was 26 years. Women from the rural area were 127 (70.55%) and from the periurban area were 53 (29.44%).

IgM-positive status for *T. gondii* was found in three women recruited from SNHRC and among the three, one was also

positive for HSV-1, whereas none of them was positive in the high-risk pregnant women recruited from PHC. Among the samples from collected SNHRC, the IgG-positive status was found 13 for *T. gondii*, 117 for Rubella virus, 82 for CMV, 93 for HSV1, 30 for HSV2, and 5 for *T. pallidum*, whereas the high-risk pregnant women group were positive 1,352,832 for *T. gondii*, Rubella virus, CMV, and HSV-1 IgG. HSV-2 and *T. pallidum* IgG were negative in all the 40 high-risk pregnant women from PHC. No statistical difference was observed in the prevalence of IgG-positive status for *T. gondii*, Rubella virus, CMV, and HSV-1 between women recruited from SNHRC and PHC [Table 1].

Among the 180 samples tested for IgG, 14 (7.77%) samples were found to be positive for *T. gondii*, 152 (84.44%) samples were positive for Rubella virus, 110 (61.11%) samples were positive for CMV, 125 (69.44%) samples were positive for HSV-1, 30 (16.66%) were positive for HSV-2, and 5 (2.77%) were positive for *T. pallidum*. The three individuals who were IgM positive for *T. gondii* and HSV-1 were in the third trimester. The IgM-positive samples for *T. gondii* and HSV-1 were also positive for IgG for respective pathogens.

Seroprevalence of TORCH-S among women was satisfied by different age group 17–25, 26–35, and >36. All IgM positives were in the age group of >36 years. Among the seven women who were in the age group of >36 years, three were positive for *T. gondii* and one was positive for HSV-1. Among IgG positives, *T. gondii* positives followed by HSV-2 were low in all three age groups compared to other pathogens tested. In the 17–25-year age group, the number of IgG positives for *T. gondii* and HSV-2 were lower compared to other pathogens. This difference was statistically significant ($P < 0.0001$). The difference in the proportion of IgG positives between 17–25 and 26–35-year age group was statistically not significant for any of the pathogens tested. The age group of >36 was too small in sample size for statistical analysis. The serological evidence of specific IgM and IgG antibodies against TORCH-S infections among pregnant women stratified by age groups is shown in Table 2, respectively.

In our study, women from the rural population were high compared to the periurban population. The seroprevalence of IgG for all the pathogens tested among the rural and periurban population is shown in Figure 1. No significant difference was

observed between rural and periurban population for any of the pathogens tested for IgG.

IgG-positive status for *T. gondii*, Rubella virus, CMV, and HSV-1 was observed in women from all the first, second, and third trimester groups. HSV-2 IgG and *T. pallidum* were seen in women in the second and third trimester only. The IgG seroprevalence for *Toxoplasma gondii*, Rubella virus, CMV, and HSV-2 was higher in the third trimester compared to other stages. The IgG-positive status for HSV-1 was higher in women at the second trimester [Table 3]. The difference, however, was not statistically significant.

One sample was exposed to all the TORCH-S agents; two samples showed mixed infections to *T. gondii*, Rubella virus, CMV, HSV-1, and HSV-2. Five samples showed mixed infections to *T. gondii*, Rubella virus, CMV, HSV-1. Seven samples showed mixed infections to Rubella virus, CMV, HSV-1, and HSV2 and 40 samples showed mixed infections to Rubella virus, CMV, and HSV-1. The IgM positives were also positive by IgG ELISA.

During the study period, among the pregnant women recruited from SNHRC, two of them developed complications later during pregnancy that leads to abortion and stillbirth. They were each in the first and second trimester, respectively. The former was positive for Rubella virus IgG and the latter was positive for Rubella virus, CMV, and HSV. HSV-2 and *T. pallidum* IgG were not observed in women at the first and second trimesters, whereas pregnant women in the second and third trimester were IgG positive for all the pathogens tested.

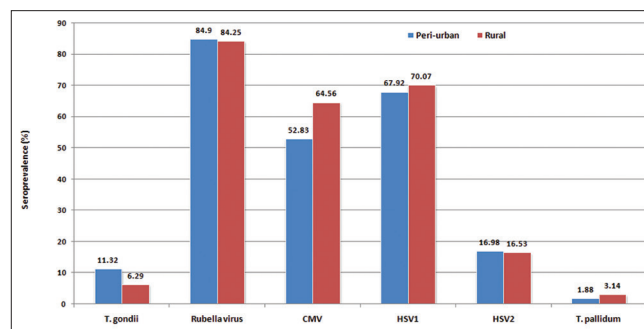


Figure 1: Seroprevalence of IgG for TORCH-S agents stratified by the domiciliary status expressed in percentages

Table 1: Seroprevalence of TORCH-S among women recruited from two centers

Study centers	<i>Toxoplasma gondii</i> , n (%)	Rubella virus, n (%)	CMV, n (%)	HSV-1, n (%)	HSV-2, n (%)	<i>Treponema pallidum</i> , n (%)
SNHRC (n=140)						
IgM	3 (2.14)	-	-	1 (0.71)	-	-
IgG	13 (9.28)	117 (83.57)	82 (58.57)	93 (66.42)	30 (21.42)	5 (3.57)
PHC (n=40)						
IgM	-	-	-	-	-	-
IgG	1 (2.5)	35 (87.5)	28 (70)	32 (80)	-	-

SNHRC: Sri Narayani Hospital and Research Centre, PHC: Primary health center, IgM: Immunoglobulin M, IgG: Immunoglobulin G, HSV-1: Herpes simplex virus-1, HSV-2: Herpes simplex virus-2, CMV: Cytomegalovirus

Table 2: Seroprevalence of TORCH-S among women stratified by different age groups

Immunoassay	Age group in years (n)	<i>Toxoplasma gondii</i> , n (%)	Rubella, n (%)	CMV, n (%)	HSV-1, n (%)	HSV-2, n (%)	<i>Treponema pallidum</i> , n (%)
IgG positives	17-25 (80)	5 (6.25)	65 (81.25)	80 (100)	77 (96.25)	8 (10)	2 (2.5)
	26-35 (93)	7 (7.52)	82 (88.17)	53 (56.98)	92 (98.92)	19 (20.43)	3 (3.22)
	>36 (7)	2 (28.57)	5 (71.42)	5 (71.42)	6 (85.71)	3 (42.85)	-
IgM positives	17-25 (80)	1 (1.25)	-	-	-	-	-
	26-35 (93)	-	-	-	1 (1.07)	-	-
	>36 (7)	2 (28.57)	-	-	-	-	-

IgM: Immunoglobulin M, IgG: Immunoglobulin G, CMV: Cytomegalovirus, HSV-1: Herpes simplex virus-1, HSV-2: Herpes simplex virus-2

Table 3: Serological evidence of specific immunoglobulin G antibodies against TORCH-S infection among pregnant women shown by gestational age

Pregnancy stage (n)	<i>Toxoplasma gondii</i> , n (%)	Rubella virus, n (%)	CMV, n (%)	HSV-1, n (%)	HSV-2, n (%)	<i>Treponema pallidum</i> , n (%)
First trimester (16)	1 (6.25)	5 (31.25)	11 (68.75)	12 (75)	-	-
Second trimester (38)	2 (1.81)	27 (71.05)	19 (50)	23 (60.52)	4 (10.52)	3 (7.89)
Third trimester (110)	11 (10)	96 (87.27)	72 (65.45)	77 (70)	26 (23.63)	2 (1.81)

Among the 180 samples, the gestational age of 16 women was not available. Of these, 14 (7.77%) were positive for Rubella, 8 (4.44%) were positive for CMV, and 13 (7.22%) were positive for HSV-1 IgG. IgG: Immunoglobulin G, CMV: Cytomegalovirus, HSV-1: Herpes simplex virus-1, HSV-2: Herpes simplex virus-2

DISCUSSION

TORCH-S infections contribute to prenatal, perinatal, and postnatal morbidity and mortality where treatment or prevention is possible for most of the pathogens.^[6] The prevalence of these infections in India has been documented in a piecemeal manner but only a very few studies exist on the prevalence of these infections as a syndromic diagnosis among pregnant women.

We looked at the seroprevalence of *T. gondii*, Rubella virus, CMV, HSV (1 and 2), and *T. pallidum* (TORCH-S) infections among pregnant women recruited from the rural and periurban population of Vellore district. The demonstration of IgM and IgG revealed the status of infections among pregnant women. Our study population included individuals recruited from our study center as well as from PHC during a special camp organized for high-risk pregnant women with BOH.

Among 180 individuals tested, *T. gondii* IgM was positive in 1.7% and HSV IgM was positive in 0.5%. On testing IgG for *T. gondii*, Rubella virus, CMV, HSV-1, HSV-2, and *T. pallidum*, a prevalence of 8%, 84%, 61%, 69%, 17%, and 3%, respectively, was observed.

In a recent study in the neighboring state of Telangana, South India, IgG seropositivity for *T. gondii*, Rubella virus, CMV, and HSV was reported to be 28%, 84%, 92%, and 61%, respectively.^[7] Our study showed a comparatively low prevalence of *Toxoplasma gondii* and CMV and the prevalence rates of Rubella virus and HSV were near equal. This indicated previous exposure to TORCH pathogens in the population. In the states of Uttar Pradesh and Maharashtra, 19%, 29%, 7%, and 8% samples were found to be IgM seropositive for *T. gondii*, CMV, HSV, and Rubella virus

among the antenatal cases ($n = 162$).^[8] In our study, the IgM positivity was <2% for *T. gondii* and <1% for HSV-1 indicating the low prevalence of current infection among these populations. In a similar study carried out at pregnant women in the first trimester, specific IgM antibodies were found to be positive in 19%, 30%, 3%, and 33% cases for *T. gondii*, Rubella virus, CMV, and HSV-2 infections, respectively. In our study, we recruited pregnant women of all three stages of pregnancy. IgG-positive status among women in the first trimester was positive for *T. gondii*, Rubella virus, CMV, and HSV-1. The *T. gondii* and HSV-1 IgM-positive women were in the third trimester. IgG-positive status for all the pathogens was positive among women at the second and third trimesters. Toxoplasmosis poses a little risk of fetal transmission (<6%) in early pregnancy, whereas the rate of transmission ranges from 60% to 81% in the third trimester.^[9] In our study, samples were collected randomly in a cross-sectional manner at one point during their regular checkups. Therefore, the prevalence data does not reflect the stages of pregnancy at which the infection was acquired. In general, pregnant women seek different health-care centers for antenatal and postnatal care. Follow-up on pregnancy outcome was also impractical in our study for this reason.

Acute and chronic *T. gondii* infection depends on serological data by the presence of IgM and IgG, respectively. However, distinguishing an acute from a chronic infection is difficult as IgM is reported to persist for several months to years following an acute infection.^[10]

T. gondii and HSV-1 IgM positives in our study were also positive for IgG. Transmission to the fetus occurs predominantly in women who acquire their primary infection during gestation, and identification of the onset of the

infection is important. Therefore, other diagnostic tools such as IgA and IgG avidity detection are suggested.^[11,12] These tests were not carried out in our study and are considered a limitation.

CRS among neonates occurs when a pregnant mother gets infected within the first 20 weeks of pregnancy. This could lead to cardiac, cerebral, ophthalmic, and auditory defects in the neonates.^[13] A systematic review carried out in India shows that 38% of pregnant women are susceptible to Rubella virus infection in India.^[14]

Determination of susceptibility to Rubella virus infection among the pregnant women in the population is therefore warranted to reveal the true risk of CRS. A recent study carried out in samples collected from different states of India reported 83% of Rubella virus IgG seropositivity among pregnant women.^[15] The overall Rubella virus IgG seropositivity in our study was 71%. Another recent study^[16] from Lucknow has reported 88% of Rubella virus IgG positivity with no difference in the age group which corroborates with our findings. In our study, CMV IgG positivity was seen in all women tested under the age group of 17–25 years. Early studies on HSV-1 and HSV-2 IgG positivity were low and significantly associated with increasing age. Little or no information in the literature exists on the prevalence of HSV-1 and HSV-2 in the population. Our study found a very high prevalence of IgG positivity for HSV-1 which is often neglected during screening for pregnant women. No studies exist on the prevalence of IgG to *T. pallidum* in pregnant women in India. We report here for the first time, a low seroprevalence of IgG of 3% in the population. The limitations of the study include lack of follow-up on pregnancy outcome among these pregnant women and detailed clinical workup. Testing newborns of these mothers were also not carried out and are considered a limitation. This indicates the necessity to screen pregnant women for TORCH-S pathogens early during the pregnancy and initiates necessary preventive and treatment options.

CONCLUSION

TORCH-S infections that include *T. gondii*, Rubella virus, *cytomegalovirus*, and HSV-1 and HSV-2 have long been known to be associated with bad obstetric outcomes. The infection presents with mild morbidity in mothers but has serious consequences to the fetus. It is therefore important to recognize the maternal infections and subsequently fetal monitoring if the infection is established. This will help the clinicians to counsel such mothers on preventive measures on the adverse fetal outcomes. Screening for pregnant women for TORCH-S agents could, therefore, reduce the incidence of adverse pregnancy and prevent birth defects.

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Conflicts of interest

There are no conflicts of interest.

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