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Original article

Prevalence of antenatal TORCH' infections by serological detection in cases of poor obstetric outcome

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ABSTRACT

The TORCH test, which is sometimes called the TORCH panel, consists of tests for antibodies to four organisms that cause congenital infections transmitted from mother to fetus. The name of the test is an acronym for the organisms detected by this panel: Toxoplasma gondii (toxoplasmosis), rubella (German measles), cytomegalovirus (CMV), and herpes simplex virus (HSV). To determine the prevalence rates of IgM and IgG to common TORCH agents in pregnant women using indirect enzyme-linked immunosorbent assay in cases of poor obstetric outcome. A total of 950 samples of sera were tested for antibodies to TORCH agents known to cause serious congenital infections: Toxoplasma gondii, rubella, cytomegalovirus (CMV), Rubella, herpes simplex viruses. In our study, out of 950 pregnant women, a total of 220 (23.1%) were positive for IgM Rubella and a total of 550 (57.8%) were positive for IgG Rubella. Toxoplasma IgM positive were 66 (6.9%), Toxoplasma IgG positive were 380 (40%), CMV IgM positive were 65 (6.8 %) CMV IgG positive were 890 (93.6 %) and 25 (2.6%) were positive for HSV II for IgM and 320 (33.6%) were positive HSV II for IgG. All antenatal cases with poor obstetric outcome should be routinely screened for TORCH as early diagnosis and appropriate intervention, will help in proper management of these cases. Some of pregnant women commonly have IgG antibodies to CMV followed by rubella, and then T. gondii, and the last one HSV.

1. Introduction

The TORCH test, which is sometimes called the TORCH panel, consists of tests for antibodies to four organisms that cause congenital infections transmitted from mother to fetus. The name of the test is an acronym for the organisms detected by this panel: Toxoplasma gondii (toxoplasmosis), rubella (German measles), cytomegalovirus (CMV), and herpes simplex virus (HSV) (1).

Although the four diseases are not particularly serious for adults who are exposed and treated, women who are become affected with any of these diseases during pregnancy are at risk for miscarriage, still birth, or for a child with serious birth defects and/or illness. Thus, this test is performed before or as soon as pregnancy is diagnosed to determine the mother's history of exposure to these organisms. The test is also performed on neonatal serum when the newborn presents with symptoms consistent with a congenitally acquired infection by one of the organisms above (2).

Poor obstetric outcome implies previous unfavorable fetal outcome in terms of two or more consecutive spontaneous abortion, history of intrauterine fetal death, intrauterine growth retardation, still births, early neonatal death and/or congenital anomalies. Causes of Poor obstetric outcome may be genetic, hormonal, abnormal maternal immune respons and maternal infection.

Recurrent pregnancy wastage due to maternal infections transmissible in utero at various stage of gestation can be caused by a wide array of organisms which include the TORCH complex (Toxoplasma gondii, Rubella virus, Cytomegalovirus, Herpes simplex virus) and other agents like Chlamydia trachomatis, Treponema pallidum, Niesseria gonorrhoeae, HIV etc. Toxoplasmosis acquired during pregnancy may cause damage to the fetus (3). Seroepidemiological studies have shown that 10-20 percent of women in childbearing age in India are susceptible to Rubella infection. Infection with Rubella during pregnancy may lead to congential malformation in 10-54 percent of cases. The infection caused by CMV in adult is usually asymptomatic but its significance is many times increased when it occurs during pregnancy. However, the rate of primary CMV infection is significantly higher for pregnant women from low socioeconomic group (4). The mother is the usual source of transmission of HSV to the fetus or newborn. Primary HSV infection during first half of pregnancy is associated with increased frequency of spontaneous abortion, still birth, and congenital malformation.

These maternal infections with adverse outcome are initially inapparent or asymptomatic and are thus difficult to diagnose on clinical grounds. Therefore, diagnosis of acute TORCH infection in pregnant women is usually established by demonstration of seroconversion in paired sera or by demonstration of specific IgM antibodies (5).

TORCH screening can be associated with both false negative and false positive results. False negative IgM tests can result from IgG antibodies to the organism binding to the antigen used in the test or from immunodeficiency syndromes that reduce the antibody response to these organisms (6). False positive test results can result from rheumatoid, autoimmune or heterophile antibodies in the mother's serum. When testing neonates, the IgG antibody levels may be detected as a result of prior infection or current maternal infection, and therefore does not mean the neonate is infected. Maternal antibodies to HSV and CMV may not adequately protect the fetus (7). This study reports the results of screening for IgG and IgM antibodies against TORCH complex in a group of patients with poor obstetric outcome

Aim of the work: In the present study our objective is to determine the prevalence rates of IgM and IgG to common TORCH agents in pregnant women using indirect enzyme-linked immunosorbent assay in cases of poor obstetric outcome.

2. Materials and methods

A total of 950 sera samples were collected from pregnant women with Poor Obstetric Outcome attending the antenatal clinic in MCH hospital. Patients included in our study were those with history of still births, habitual abortions, intrauterine growth retardation and neonatal deaths.

Toxoplasma gondii, Rubella virus, CMV and HSV-II IgM and IgG antibodies were tested of serum by sandwich ELISA (BIOKIT,S.A.,BARCELONA-SPAIN) The procedures were:

1) 10 ul of serum into 1 ml of diluent. Use only the number of strips required for the test. Reserve 8 wells for blank and controls. Use two wells for negative and high positive control and three wells for low positive control. Pipette 100 ul of each control and each diluted sample to the corresponding wells. Leave a well empty for the substrate blank. 2) Cover the micro plates with an adhesive seal and incubate for 1 hour at 37C.3) Remove and discard the adhesive seal. Aspirate the content of the wells and fill them completely (approximately 350 ul) with the diluted washing solution. Repeat the process of aspiration and washing 3 times. After the last washing blot the microplate on absorbance tissue to remove any excess liquid from the wells. 4) Transfer 100 ul of diluted conjugate into each well of microplate, except the blank. Avoid bubbles upon dilution. 5) Cover the micro plate with an adhesive seal and incubate for 1 hour at 37C.6) During the last 5-10 minutes of this incubation prepare the substrate-chromogen solution. 7) Remove and discard the adhesive seal. Aspirate the content of the wells and fill them completely (approximately 350 ul) with the diluted washing solution. Repeat the process of aspiration and washing 3 times. After the last washing blot the micro plate on absorbance tissue to remove any excess liquid from the wells. 8) Add 100 of substrate - TMB solution to each well, including the blank. 9) Incubate for 30 min. at room temp. (20- 25C).10) Stop the reaction by adding 100 ul of stopping solution.11) Blank the reader at 450 nm with the blank well and read the absorbance of each well. It is recommended to read the absorbance at 620- 630 nm, within 30 min.

2.1. Interpretation of the test

TORCH index of each determination was calculated by dividing the absorbance value of each sample by the absorbance of cut-off value.

*Positive : ratio absorbance / cut-off > or = 1.0

*Negative: ratio absorbance / cut-off < 0.9

*Equivocal: ratio absorbance / cut-off > or = 0.9 < 1.0

3. Results

In our study, out of 950 pregnant women with Poor Obstetric Outcome, a total of 220 (23.1%) were positive for IgM Rubella and a total of 550 (57.8%) were positive for IgG Rubella. Toxoplasma IgM positive were 66 (6.9%), Toxoplasma IgG positive were 380 (40%), CMV IgM positive were 65 (6.8 %) CMV IgG positive were 890 (93.6 %) and 25 (2.6%) were positive for HSV II for IgM and 320 (33.6%) were positive HSV II for IgG . The table shows the results of IgG and IgM antibody assays in all patients included in the study.

Table 1
this table shows the results of IgG and IgM antibody assays in all patients included in the study:

Serological Tests	No. of positive	%	No. of negative	%
Toxoplasma Gondii				
IgM	66	6.9	884	93.0
IgG	380	40	570	60.0
Rubella				
IgM	220	23.1	730	76.8
IgG	550	57.8	400	42.1
CMV				
IgM	65	6.8	885	92.6
IgG	890	93.6	60	6.3
HSV II				
IgM	25	2.6	925	97.3
IgG	320	33.6	630	66.3

Also, we found that, there was mixed infection was noted in 23 out of 66 patients (34.8%) in association with Toxoplasma IgM antibodies in our study. Out of 23 patients of mixed infection 19 were with Rubella, three with CMV and one each with Rubella plus CMV and CMV plus HSVII.

4. Discussion

In the present study, Toxoplasma gondii, which is a known etiological agent in recurrent pregnancy wastages, was found in 6.9 % pregnant women with Poor Obstetric Outcome as reported earlier(8). Congenital transmission of toxoplasmosis is known to occur during acute phase of maternal infection. IgM antibodies were found in 12% patients in other studies (9). In anther study, Overall IgG seroprevalence rate of toxoplasmosis was 45%. Only seven women (3.3%) were found to have IgM antibodies and only two of these showed low IgG avidity indicating recent infection of ≤4 months duration. One woman aborted spontaneously at her fourth month of gestation. In remaining five women the recent infection could successfully be excluded by IgG avidity testing. All these women had uneventful pregnancy (13).

In anther study, they found that, from four hundred and sixty-two maternal TORCH tests were performed. Of those, TORCH tests were also performed on fetal samples (amniotic fluid or fetal blood) in 67 cases. Fourteen fetal tests without maternal testing were identified; making the total number of patients tested 476. There were 11 cases of maternal CMV infection (2.3%), 10 cases of fetal CMV infection, and none of the other viruses. Indications for testing included fetal hyperechogenic bowel, hydrops, cerebral ventriculomegaly, echogenic foci, oligohydramnios, polyhydramnios, and IUGR. The most common findings to be actually associated with fetal infections were hyperechogenic bowel, ascites, cardiomegaly, and oligohydramnios. No cases were associated with polyhydramnious, while both IUGR and ventriculomegaly were always associated with other more relevant features (14).

Seroepidemiological studies have shown that 10-20 percent of women in childbearing age in India are susceptible to Rubella infection. Infection with Rubella during pregnancy may lead to congential malformation in 10-54 percent of cases. The infection caused by CMV in adult is usually asymptomatic but its significance is many times increased when it occurs during pregnancy. However, the rate of primary CMV infection is significantly higher for pregnant women from low socioeconomic group (4).

If it is found that, episodes of increased incidence of Rubella are reported to occur every 3-4 years. Since 10-20% of women in child bearing age are susceptible to Rubella, (10) increased incidence of Rubella will lead to increased reporting of pregnant women with Rubella infection. In the present study, 23.1% pregnant women were positive for Rubella IgM as has been reported earlier (11). The observation therefore suggests an increased incidence of Rubella infection in pregnant women.

Primary infection with HSV II acquired by women during pregnancy accounts for half of the morbidity and mortality from HSV II among neonates. The other half results from reactivation of old infection. Seropositivity rate of HSV IgM among the poor obstetric outcome patients of our study was 2.6%. HSV in asymptomatic women with recurrent infection during pregnancy was found to be 0.6-3% previously (15).

Primary CMV infection in pregnancy has a higher incidence of symptomatic congenital infection and fetal loss. This infection, being asymtomatic in adults, is difficult to diagnose clinically (12). Demonstration of IgM antibodies is indicative of primary infection. The present study shows, seropositive rate of 6.8 % for CMV IgM in women with poor obstetric outcome as has been reported previously.

The need of serological evaluation of CMV specific IgM during pregnancy has been supported by various investigators (13).

5. Conclusion

We concluded that all antenatal cases with poor obstetric outcome should be routinely screened for TORCH as early diagnosis and appropriate intervention will help in proper management of these cases. Some of pregnant women commonly have IgG antibodies to CMV followed by rubella, and then T. gondii, and the last one HSV.

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