



Study to Evaluate Association of Torch Infection with Bad Obstetric History in Pregnant Women at Sms Medical College and Attached Group of Hospitals, Jaipur

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Congenital infections are transmissible in utero and it can lead to serious foetal outcomes. These infections can be early detected in pregnant women with bad obstetric history for better foetal outcomes.

Aim of the Study was to evaluate the association of TORCH infection with bad obstetric history among pregnant women.

Study Design: Observational and comparative study

Place and Duration of Study: Central laboratory, Department of Microbiology, SMS Medical College, Jaipur between April 2020 and September 2021.

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Methodology: 260 blood samples of pregnant women (130 with bad obstetric history and 130 pregnant women without bad obstetric history) were collected. and tested for the presence of IgM and IgG antibodies against *Toxoplasma gondii*, Rubella virus, Cytomegalovirus by Chemiluminescence and Herpes simplex virus using ELISA kits.

Results: Overall TORCH IgM seropositivity in high-risk pregnant women was 17.19%. In pregnant women with bad obstetric history, IgM Seropositivity for *Toxoplasma gondii* was 3.84% (*P* value .02), rubella 2.34% (*P* value .30), Cytomegalovirus 5.47% (*P* value .08), and 6.25% (*P* value .56) for Herpes-1 and 2 infections and IgG seropositivity for toxoplasma, rubella, cytomegalovirus and herpes virus was 16.41% (*P* value .001), 93.75% (*P* value .11), 98.44% (*P* value .55), 48.44% (*P* value .53) respectively. In pregnant women without bad obstetric history, IgM and IgG seropositivity for toxoplasma, rubella, cytomegalovirus and herpes virus was 0/0.77%, 0.76/97.69%, 1.53/99.23% and 4.61/44.62% respectively. The average age of the study population was 27.13 years.

Conclusion: As TORCH infections are transmissible in-utero in all the stages of pregnancy and contributes in neonatal and infant deaths, so early diagnosis and appropriate interventions necessary which help in proper management of the pregnant women.

Keywords: Pregnant women; bad obstetric history; congenital infections; IgM and IgG seropositivity; seroprevalence; TORCH infections; TORCH screening.

ABBREVIATIONS

TORCH : *Toxoplasma, Rubella, Cytomegalovirus, Herpes simplex virus, others*
BOH : *Bad obstetric history*
CMV : *Cytomegalovirus*
HSV : *Herpes simplex virus*
IUFD : *Intra uterine foetal death*
IUGR : *Intra uterine growth retardation*
Ig : *Immunoglobulin*
ELISA : *Enzyme linked immunosorbent assay*
CLIA : *Chemiluminescence assay*

1. INTRODUCTION

Congenital infections can cross the placenta and leads to damage to the foetus in utero. Its transmission during pregnancy causes neonatal infections [1-6]. Apart from stillbirths, miscarriage and neonatal deaths, congenital infections account for 2% to 3% of all congenital anomalies [7].

Following principles are included in the development of congenital defects:

- (1) Viral infections in pregnant women and transmission in foetus.
- (2) The pregnancy trimester in which infection occur.
- (3) The ability of the virus to cause damage to the foetus directly (by infection of the foetus) or indirectly (by infection of the mother), resulting in an altered foetal condition.

Bad obstetric history (BOH) can be caused due to genetic, hormonal, abnormal maternal immune response and maternal infections [8-15].

Adverse foetal outcomes such as two or more consecutive spontaneous abortions, intrauterine growth retardation (IUGR), early neonatal death, history of intrauterine foetal death (IUFD), congenital anomalies and/or stillbirth indicate bad obstetric history [16-23]. Recurrent pregnancy wastage due to maternal infections transmissible *in utero* in any trimester of gestation can be caused by a wide array of organisms which include the TORCH complex and other agents like *Chlamydia trachomatis*, *Treponema pallidum*, *Niesseria gonorrhoeae*, Human Immunodeficiency Virus (HIV) [24].

The acronym **TORCH** was coined by Immunologist **Andre Nahmias** for *Toxoplasma gondii* parasite, Rubella virus, Cytomegalovirus and Herpes Simplex virus type I & II [25]. In 1975, **Harold Fuerst** proposed adding syphilis to the list and revising the acronym into **STORCH**. In 1975, **Roger Brumback** coined TORCHES which replaced STORCH. The 'O' in TORCH stands for '**Others**' which include syphilis, parvovirus, coxsackie virus, listeriosis, hepatitis virus, varicella-zoster virus, *Trypanosoma cruzi*, enterovirus, human immunodeficiency virus (HIV), and Zika virus [7].

Epidemiologic risk factors for congenital infection may be exposure and consumption of raw meat, failure to wash home garden product, high risk sexual behaviour, history of sexually transmitted disease (STI), occupational exposure [26-30].

Toxoplasma gondii is an obligate intracellular parasite which is acquired by ingestion of food or water contaminated by infected cat feces or by undercooked meat containing tissue cyst of toxoplasma. Infection may be congenital if women is infected during or just prior to pregnancy [25].

Rubella virus is the etiological agent of “**German measles or Third disease**”, is a viral illness which belongs to the family *Togaviridae* and genus *Rubivirus* [31]. Infection in first trimester will lead to miscarriage and in later trimester, it may lead to intrauterine foetal death, cataract, mental retardation hearing impairment and cardiac defect collectively known as **congenital rubella syndrome** (incidence 0.6-2 cases/1000 live birth) [32].

Cytomegalovirus is species-specific, ubiquitous, family *Herpes virus*. It is transmitted by direct contact with breast milk, blood, urine, genital secretion and saliva [33].

Congenital Cytomegalovirus infection (CCMV) is the most common intrauterine infection and affects 0.3%–2.5% of live births. It is estimated that in the case of a primary infection, the risk of mother-to-child transmission is 24%–75%, while during reactivation of CMV infection, the risk is only 1% [34].

Herpes Simplex Virus (HSV) is a ubiquitous, enveloped virus having double stranded DNA, belonging to the family *Herpesviridae*. It is transmitted across mucosal membranes and non-intact skin, that migrate to nerve tissues, where they persist in a latent state. Herpes virus is of 2 types- HSV-1 & HSV-2 [35].

Acute TORCH infection is the major cause of peri-natal and post-natal morbidity and mortality. These infections not only lead to single or repeated foetal loss but also lead to delayed complications to both pregnant women and foetus [36]. Severity of these infections depends on the gestational age at the time of infection, infectivity of organism, placental damage [37]. TORCH screening in antenatal women is recommended for early detection and better treatment of congenital infection which can prevent complications in both mother and foetus [38].

1.1 Aims and Objectives

Aims: To evaluate the association of TORCH infection with bad obstetric history among pregnant women.

Objective: a) To determine the difference in proportion of cases who are TORCH positive among pregnant women with and without bad obstetric history.

b) To describe the microbiological profile of TORCH positivity in both cases and control groups.

2. MATERIALS AND METHODS

Study design: It was undertaken as an observational and comparative study at Sawai Man Singh Medical College and Attached group of Hospitals, Jaipur from April 2020 to September 2021.

Study population: A total of 260 subjects were included for the study. **Group I** comprised of 130 pregnant women with bad obstetric history (cases) with the age range of 18-40 years. **In Group II** age matched control group of 130 healthy pregnant women with previous normal obstetric history were included. History was collected in the specially designed data collection form.

Inclusion criteria: Pregnant women of reproductive age group with bad obstetric history (cases) or without bad obstetric history (control).

Exclusion criteria: Cases with hypertension, Diabetes mellitus, syphilis, eclampsia of pregnancy, Rh incompatibility, HIV positive pregnant women.

2.1 Sample Collection and Processing

3-5 mL blood sample was collected under aseptic precautions and was allowed to clot and centrifuged at 3000 rpm for 10 minutes. Serum samples were stored in small screw capped plain vials at 2 to 8 degrees Centigrade until processed. The Samples were tested for the presence of IgM and IgG antibodies against *Toxoplasma gondii*, Rubella virus, Cytomegalovirus by Chemiluminescence (Elecsys IgG/IgM kit by Roche Diagnostic) and Herpes simplex virus using ELISA kits (**ENZYWELL HERPES ELISA kit**).

2.2 Principle of Chemiluminescence Immunoassay (CLIA)

In the presence of complementary antigen and antibody, the paratope of the antibody binds to the epitope of the antigen to form an antigen-antibody or an immune complex. Estimating the levels of such immune complex by the use of labelled antibodies from the basis of CLIA. It involves use of stationary solid particles coated either with the antigen or antibody of interest. Post incubation, which ensures intact immune complexes are formed, substrate is added. This results in generation of light, the intensity of which is directly proportional to the number of labelled complexes present and which indirectly aids in qualification of the analysis of interest. The intensity of light is measured in terms of relative light units (RLU).

- In the first step, serum antibodies bind with the biotinylated and ruthenium labelled antigens to form an immune complex.
- The immune complex then reacts with streptavidin-coated micro bead through the action of the biotinylated antigen.
- After the second incubation, the reaction mixture containing the immune complexes were transported into the measuring cell; and they were magnetically entrapped on the working electrode, and the unbound reagent and the samples were washed away by Procell.

In the electrochemiluminescence reaction, the conjugate was a ruthenium-based derivative and the chemiluminescent reaction was electrically stimulated to produce light. The amount of light produce was directly proportional to the amount of analyse in the sample.

2.3 Principle of Herpes IgM/IgG ELISA Test

- The test is based on the Enzyme linked Immunosorbent Assay technique (ELISA).
- The antigen, composed of purified and

inactivated Herpes Simplex Virus types 1 and 2, is bound to the solid phase (8-well strips).

- The specific immunoglobulins are bound to the antigen through incubation with dilute human serum.
- After washings to eliminate the proteins which have not reacted, incubation is performed with the conjugate, composed of human IgG monoclonal antibodies labelled with peroxidase.
- The unbound conjugate is eliminated and the peroxidase substrate is added.
- The colour which develops is proportional to the concentration of specific antibodies present in the serum sample.

3. RESULTS

In pregnant women with bad obstetric history (cases), IgM and IgG antibodies for Toxoplasma, rubella virus, Cytomegalovirus and Herpes Simplex Virus 1 and 2 were 3.9/16.41%, 2.34/93.75%, 5.47/98.44% and 6.25/48.44% respectively, while in pregnant women without bad obstetric history (control), IgM and IgG antibodies for Toxoplasma, rubella virus, Cytomegalovirus and Herpes Simplex Virus 1 and 2 were 0/0.77%, 0.77/97.7%, 1.54/99.23% and 4.61/44.62% respectively (Tables:1 & 2). So, according to our study, significant difference in IgM and IgG seropositivity was found in toxoplasma infection as the *P* valve was less than .05. There was no significant difference found in rubella virus infection, CMV and herpes simplex virus infection in cases and control group. Similarly, in table 3, significant difference in either IgM or IgG seropositivity was found in pregnant women with bad obstetric history in case of toxoplasma gondii infection (*P* value .001). in cases of only IgM seropositivity, significant difference was found in TORCH seropositivity as *P* value found .01.

Comparative result of TORCH infections in cases and control groups was shown in Tables 1 and 2.

Table 1. Comparison of TORCH infections based on IgM seropositivity in cases (with bad obstetric history) and controls (with normal obstetric history) (N=260)

| TORCH infections | Cases | Controls | χ^2 value | df | <i>P</i> value |
|----------------------|-----------|-----------|----------------|----|----------------|
| Toxoplasma gondii | 5(3.9%) | 0(0%) | 5.178 | 1 | .02 |
| Rubella virus | 3(2.34%) | 1(0.769%) | 1.048 | 1 | .30 |
| Cytomegalovirus | 7(5.469%) | 2(1.538%) | 2.959 | 1 | .08 |
| Herpes simplex virus | 8(6.25%) | 6(4.61%) | 0.336 | 1 | .56 |

df – degree of freedom

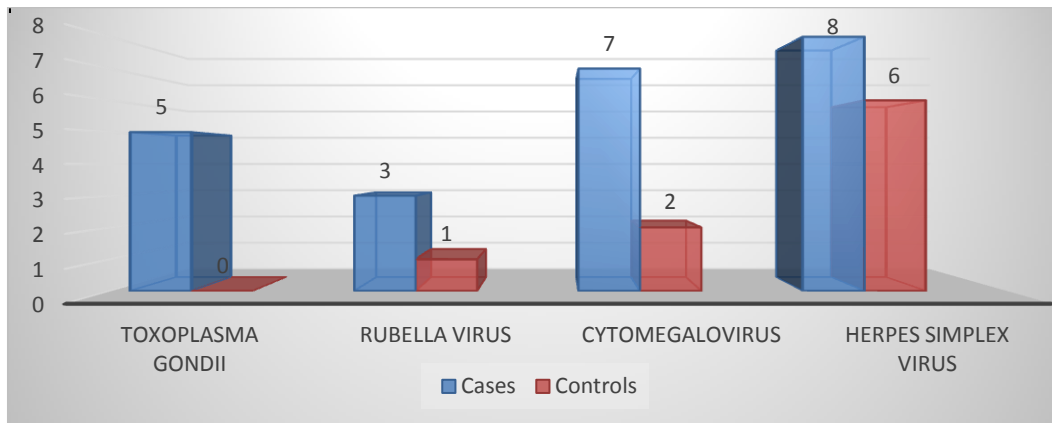


Fig. 1. Comparison of TORCH infections based on IgM seropositivity in cases (with bad obstetric history) and controls (with normal obstetric history)

Table 2. Comparison of TORCH infections based on IgG seropositivity in cases (with bad obstetric history) and controls (with normal obstetric history) (N=260)

| TORCH Infections | Cases | Controls | χ^2 value | df | P value |
|----------------------|-------------|-------------|----------------|----|---------|
| Toxoplasma gondii | 21(16.41%) | 1(0.77%) | 20.22 | 1 | .001 |
| Rubella virus | 120(93.75%) | 127(97.69%) | 2.46 | 1 | .11 |
| Cytomegalovirus | 126(98.44%) | 129(99.23%) | 0.353 | 1 | .55 |
| Herpes simplex virus | 62(48.44%) | 58(44.62%) | 0.379 | 1 | .53 |

df – degree of freedom

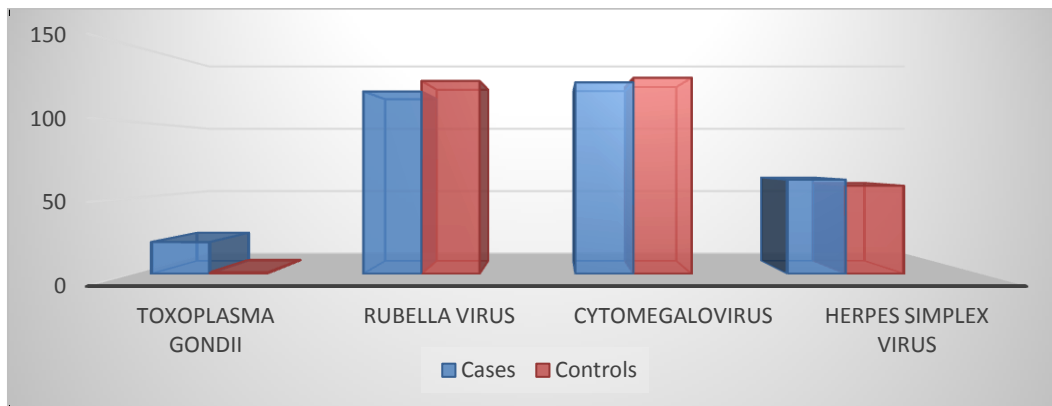


Fig. 2. Comparison of TORCH infections based on IgG seropositivity in cases (with bad obstetric history) and controls (with normal obstetric history)

Table 3. Comparison of TORCH infections based on either IgM or IgG seropositivity in cases (with bad obstetric history) and controls (with normal obstetric history) (N=260)

| TORCH Infections | Cases | Controls | χ^2 value | df | P value |
|---------------------------------|-------------|-------------|----------------|----|---------|
| Toxoplasma gondii | 25(19.53%) | 1(0.769%) | 25.05 | 1 | .001 |
| Rubella virus | 120(93.75%) | 127(97.69%) | 2.46 | 1 | .11 |
| Cytomegalovirus | 126(98.44%) | 129(99.23%) | 0.353 | 1 | .55 |
| Herpes simplex virus | 67(52.34%) | 61(46.92%) | 0.76 | 1 | .38 |
| TORCH infection (including all) | 130 (100%) | 130(100%) | 0.02 | 1 | .90 |
| TORCH infection (only IgM) | 22(17.19%) | 9(6.92%) | 6.42 | 1 | .01 |

Table 4. Descriptive statistics of quantitative variables

| Variable | Mean | SD |
|-------------|-------|------|
| Age (years) | 27.13 | 4.34 |

So, the mean age for our study was 27.13 with standard deviation 4.34.

Out of 130 pregnant women with bad obstetric history, 94.61% pregnant women having history of abortions followed by IUFD (2.3%), IUGR (2.3%), early neonatal death (1.53%), congenital

malformations (1.53%) and still birth (1.53%) were included in Table 5.

In abortion cases, seropositivity found most commonly found in cytomegalovirus infection (5.38%) and herpes simplex virus (5.38%) followed by *Toxoplasma gondii* infection (3.07%) and rubella virus infection (2.3%). IgM and IgG seropositivity of Toxoplasma, Rubella and Cytomegalovirus and Herpes simplex virus according to various obstetrics losses are shown in Table 6.

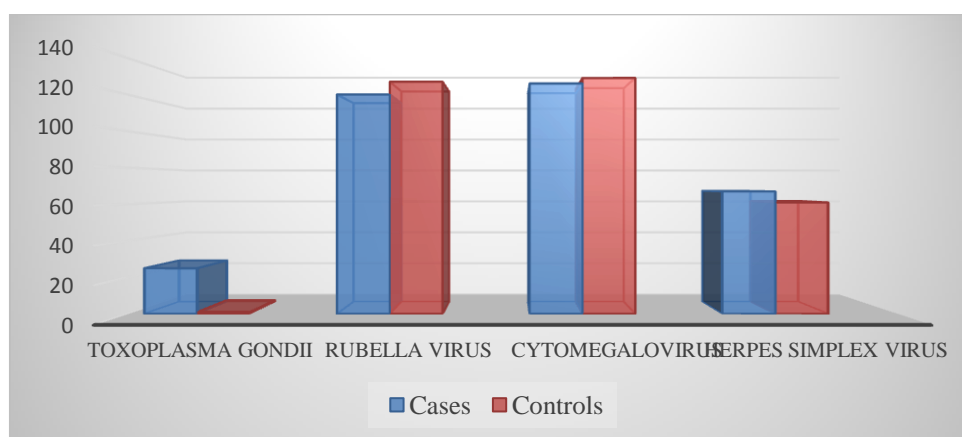


Fig. 3. Comparison of TORCH infections based on either IgM or IgG seropositivity in cases (with bad obstetric history) and controls (with normal obstetric history)

Table 5. Distribution of cases under study on basis of bad obstetric history

| Sr. No. | Post obstetric outcome | Number of cases studied and (%) |
|---------|--------------------------|---------------------------------|
| 1. | Abortion | 123 (94.61%) |
| 2. | IUFD | 03 (2.3%) |
| 3. | IUGR | 03 (2.3%) |
| 4. | Early neonatal deaths | 02 (1.53%) |
| 5. | Congenital malformations | 02 (1.53%) |
| 6. | Stillbirth | 02 (1.53%) |

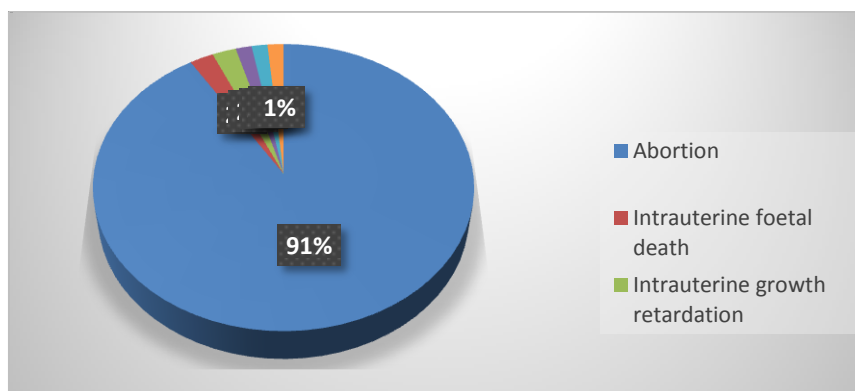


Fig. 4. Distribution of cases under study on basis of bad obstetric history

Table 6. Correlation of IgM and IgG seropositivity of TORCH infection with bad obstetric history

| BOH | Tm | Rm | Cm | Hm | Tg | Rg | Cg | Hg |
|-------------------------|------------|------------|------------|------------|-------------|----------------|----------------|--------------|
| Abortion | 04 (3.07%) | 03 (2.3%) | 07 (5.38%) | 07 (5.38%) | 21 (16.15%) | 125 (96.15%) | 125 (96.15%) | 62 (47.69%) |
| IUGR | 00 | 00 | 00 | 00 | 00 | 02 (1.53%) | 02 (1.53%) | 01 (.76%) |
| IUFD | 00 | 00 | 00 | 00 | 01 (.76%) | 03 (2.3%) | 03 (2.3%) | 02 (1.53%) |
| Early neonatal death | 00 | 00 | 00 | 01 (0.76%) | 00 | 02 (1.53%) | 02 (1.53%) | 01 (.76%) |
| Congenital malformation | 00 | 00 | 01 (.76%) | 00 | 01 (.76%) | 05 (3.84%) | 05 (3.84%) | 02 (1.53%) |
| Still birth | 00 | 01 (.76%) | 00 | 00 | 00 | 02 (1.53%) | 02 (1.53%) | 01 (.76%) |
| Total | 04 (3.07%) | 04 (3.07%) | 08 (6.15%) | 08 (6.15%) | 23 (17.69%) | 139 (106.92 %) | 139 (106.92 %) | 69 (53.07 %) |

Table 7. Association of socio-economic status with obstetric history (N=260)

| Attributes | Levels | Socio-economic status | | | χ^2 value | df | P value |
|-------------------|---------------|------------------------------|---------------|--------------|----------------|-----------|----------------|
| | | Lower | Middle | Upper | | | |
| Obstetric History | Bad | 10 (7.81%) | 111((86.72%) | 7(5.47%) | 1.24 | 2 | .538 |
| | Normal | 8(6.15%) | 118(90.77%) | 4(3.08%) | | | |

Table 8. Association of socioeconomic status with TORCH infection seropositivity (IgM) in pregnant women with bad obstetric history

| Attributes | Level | Socioeconomic status | | |
|-------------------|--------------|-----------------------------|---------------|--------------|
| | | Lower | Middle | Upper |
| TORCH infection | Positive | 02 (1.53 %) | 21 (16.15 %) | 01 (0.76 %) |
| | Negative | 08(6.15 %) | 90(69.23 %) | 06 (4.61 %) |

According to our results, seropositivity found maximum in case of middle socio-economic class people. These people are more aware about health care facility which should be provided during pregnancy. Because of lack of proper education system, lower class people do not come for regular antenatal check-up and TORCH screening. So, seropositivity found less in case of them as a smaller number of pregnant women attended antenatal clinic and there was no significant association between bad obstetric history and socioeconomic status of pregnant women as P value = .53 (Tables: 7&8).

4. DISCUSSION

TORCH infections are associated with adverse foetal outcomes like recurrent abortions, IUFD, still birth, IUGR, congenital malformations and other reproductive failures especially during the first trimester of the pregnancy as placental transmission of IgG antibodies against these microorganisms from mother to foetus is more during first trimester and foetal immune system is unable to resist these microorganisms [39-44].

In our study, 130 pregnant women with bad obstetric history (cases) and 130 pregnant women without bad obstetric history (controls) were included. Out of 130 pregnant women with bad obstetric history 95% pregnant women having history of abortions which was found most commonly in first trimester of pregnancy, followed by IUFD (2.3%), IUGR (2.3%), congenital malformations (1.53%) and stillbirth (1.53%).

According to our study, Toxoplasma specific IgM and IgG antibodies in pregnant women with bad obstetric history were 3.84% (5/130) and 16.41% (21/130) respectively. Our study coincides with the studies conducted by Thapliyal N et al. [45], Tamer et al. [46], Nabi SN et al. [47], Padmavathy M et al. [25], Shrivastava G et al [48], Bagheri Josheghani S et al. (2015), Kusuma Nellimarla et al. (2017), Kumar R et al [32], Sahu S et al [49], Baghel S et al [50] showing IgM and IgG seropositivity as 20/55%, 0.4/48.3 %, 0.9/23.42%, 5.8/8%, 30.15/9.5%, 3.8/37.5%, 20/30%, 2.66/5.33%, 0.7/38.3%, 0/5.5% respectively.

Similarly, the Toxoplasma specific IgM and IgG antibodies in pregnant women without bad obstetric history (controls) was 0% and 0.76% respectively which was comparable with the study of Surpam RB et al. [51], Kumari N et al

[52], Guddy et al. [53], Saxena N et al. (2015), Rasti S et al. (2016), Mustafa M et al [54] in which the IgM seropositivity was 1.33%, 16.7%, 0%, 1.50%, 2.85%, 0% respectively.

In our study, Rubella IgM and IgG antibodies were 2.34% (3/130) and 93.75% (120/130) respectively. Our study is comparable with studies done by Tamer et al. [46], Nabi SN et al. [47], Padmavathy M et al. [25], Shrivastava G et al. [48], Bagheri Josheghani et al. (2015), Kusuma Nellimarla et al. (2017), Kumar R et al. [32], Sahu S et al. [49], Baghel Set al. [50] IgM and IgG seropositivity was 0.2/96.1%, 6.3/81.08%, 4.6/91.75%, 6.34/23.80%, 0/92.5%, 5 /46.6%, 6.66 /84%, 0.5/68.4%, 6/75.5% respectively.

IgM and IgG antibodies for rubella virus in pregnant women without bad obstetric history in our study was 0.76% and 97.69% respectively which was comparable with the study of Surpam RB et al. [51], Kumari N et al. [52], Saxena N et al. (2015), Rasti S et al. (2016), Mustafa M et al. [54] in which the IgM seropositivity was 1.33%, 0%, 0%, 2.04%, 2.85% respectively.

In our study, Cytomegalovirus IgM and IgG antibodies were 5.47% (7/130) and 98.44% (126/130) respectively, which was comparable with studies of Thapliyal N et al. [45], Tamer et al. [46], Nabi SN et al. [47], Padmavathy M et al. [25], Shrivastava G et al. [48], Bagheri Josheghani S et al. (2015), Kusuma Nellimarla et al (2017), Kumar R et al. [32], Sahu S et al [49], Baghel S et al. [50] IgM and IgG seropositivity was 26.7/93%, 0.7/ 96.4%, 0.9/91.49%, 9.2/96.4%, 7.9/15.8%, 5/98.8%, 6.6/93%, 9.33/82.66%, 1.7/57.2%, 4/56% respectively.

IgM and IgG antibodies against cytomegalovirus infection in pregnant women without bad obstetric history was 1.53% and 99.230% respectively. The IgM antibodies against this infection was comparable with the study of Surpam RB et al. [51], Kumari N et al. [52], Saxena N et al. (2015), Rasti S et al. (2016), Mustafa M et al. [54] which were 1.33%, 0%, 2.85%, 2%, 12.2%, 14.2% respectively.

In our study, Herpes Simplex Virus IgM and IgG antibodies were 6.25% (8/130) and 48.44% (62/130) respectively. Seropositivity for herpes simplex virus of our study is comparable with the study of Thapliyal N et al. [45], Nabi SN et al [47], Padmavathy M et al. [25], Josheghani BS et al. [55], Nellimarla K et al. (2017), Kumar R et al

[32], Sahu S et al. [49], Baghel S et al. [50], IgM and IgG seropositivity was 26.7 /73%, 2.7 /87.29%, 2.3 /5.8%, 7.5/91.3%, 0/6.6%, 8 /38.66%, 2 /21.1%, 0/14.5% respectively.

Herpes simplex virus specific IgM and IgG antibodies in pregnant women without bad obstetric history was 4.61% and 44.62% respectively which was comparable with the study of Surpam RB et al. [51], Kumari N et al. [52], Saxena N et al. (2015), Rasti S et al. (2016), Mustafa M et al. [54] in which the IgM seropositivity was 4%, 0%, 5.71%, 3%, 14.2% respectively.

According to our study, IgM seropositivity in pregnant women with bad obstetric history found maximum in case of herpes simplex virus i.e., 6.25%, followed by 5.47%, 3.84% and 2.34% in CMV, toxoplasma and rubella respectively, but significant association of bad obstetric history in pregnant women was found in case of toxoplasmosis as *P* value found less than .05 in both IgM and IgG seropositivity of TORCH infection. There was no significant difference in rubella, CMV and herpes infection with bad obstetric history in pregnant women.

In India, most of the women of child-bearing age belonging to lower socio-economic group and reside in rural/tribal areas. They come under high-risk pregnancy group since, they are exposed to a wide range of infections due to poor environmental conditions and lack of good personal hygienic practices, more animal contact, giving more time for agriculture work as farming is the main occupation in India which increases the exposure to soil contaminated with the infected cat faces, poor sanitary conditions and lack of awareness because of poor education system. These infections are more common in people living in small area (overcrowding), day care centre which promote their transmission from one person to another.

5. CONCLUSION

TORCH infections are transmissible in-utero in all stages of pregnancy but mostly in first trimester. So, all the pregnant women should be screened for TORCH infection during her first antenatal visit. Early diagnosis and appropriate interventions help in proper management of these congenital infections.

ETHICAL APPROVAL

The study protocol was approved by ethics committee.

CONSENT

As per international standard or university standard, patient's consent has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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