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Seropositivity of ToRCH infection in Pregnant Women in Tertiary Care Hospital

Vishakha Asopa[1], Eshank Gupta[1], Anita Kumari Gupta[1], A. S. Dalal[2] and Prabhu Prakash[1*]

Department of Microbiology, Dr SNMC, Jodhpur, India
Department of Microbiology, GMCH, Udaipur, India
**Corresponding author*

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A B S T R A C T

ToRCH is an acronym which stands for *Toxoplasma gondii, Rubella virus, Cytomegalovirus (CMV), Herpes simplex virus-II (HSV-II)* and other agents like *Zika virus, HIV, Treponema pallidum, Niesseria gonorrhoeae*, etc are the major cause of bad obstetric history (BOH). These pathogens usually cause only asymptomatic or mild infections in mother, but can cause much more serious consequences in fetus. The present study was aimed to find seroprevalence in pregnant population to explore the burden of ToRCH [toxoplasma, rubella virus, cytomegalovirus (CMV) and herpes simplex virus (HSV)] infection in southern Rajasthan. This was a prospective study conducted in a tertiary care center, Udaipur .The study period was carried over one year. Total 111 non duplicate blood samples were collected from pregnant women. Seropositivity (IgM/IgG) of ToRCH infection in pregnant women were detected by Chemiluminescence Immuno assay method. *Herpes simplex virus -2* was detected by Enzyme linked immunosorbent assay. Out of 111 screened pregnant women, overall seropositivity (IgM/IgG) for *Toxoplama gondii* was 03 (2.70%), *Rubella* 43 (38.74%), *Cytomegalovirus* 52(46.85%) and *Herpes simplex –II* was 02(1.80%). Seropositivity of IgM for specific ToRCH agents were as follows: *Toxoplama* 02(1.80%), *Rubella* was 15(13.51%), *Cytomegalovirus* was 20(18.01%) and *Herpes simplex –II* was 01 (0.90%). The higher seropositivity (IgM/IgG) was found between 20 to 40 years. Higher seropositivity rates were observed in pregnant women who were in first trimester. It is recommended that all pregnant women with BOH should be routinely screened for ToRCH agents at the first antenatal visit as ToRCH infections play a major role in adverse fetal outcome. Awareness amongst clinicians and patients about ToRCH infections and their consequences should developed.

Introduction

Screening of pregnant women for the presence of infectious disease is critical in helping to ensure normal embryogenesis and a healthy birth. Between 2% to 3% of all congenital anomalies are attributed to perinatal infection (1).The maternal infections that are transmissible in-utero at several stages of the pregnancy can be caused by ToRCH complex.

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Cytomegalovirus (CMV), Herpes simplex virus-II (HSV-II) and other agents like *Zika virus, HIV, Treponema pallidum, Niesseria gonorrhoeae,* etc are the major cause of bad obstetric history (BOH).These pathogens usually cause only asymptomatic or mild infections in mother, but can cause much more serious consequences in fetus(2).

The gestational age of the fetus influenced the degree of severity. The placenta forms a barrier between mother and fetus during the first trimester of pregnancy that protects the fetus from the humoral and cell mediated immunological response. Although, the fetus gets immunity from mother, they are seriously infected by these viruses due to lack of immunity after the first trimester of pregnancy (3). All the infections have their own causative agent and generally they spread through poor hygienic conditions, contaminated water, food, soil and airborne respiratory droplet. The social and reproductive maladjustment because of repeated pregnancy wastage, cost of treatment and morbidity caused to the infant make ToRCH group of infections a major concern.

Toxoplasmosis is a parasitic infectious disease caused by a protozoan *Toxoplama gondii,* which is transmitted to human through ingestion of food or water contaminated with cat feces or eating undercooked meat of the infected sheep, goat, pig, cow and other avian species. The infection is carried to the infant through mother's placenta, and cause infections of the eye or the central nervous system (CNS)(4).

Rubella virus is a single stranded RNA virus of paramyxovirus group. *Rubella* virus spreads from person to person by respiratory droplets via upper respiratory mucosa. Approximately 30 % to 40% fetuses of women who contact with *Rubella* during the first trimester of pregnancy will be adversely affected by the virus. The *Rubella* virus readily invades the placenta and the fetus during gestation and it causes ear, occular, cardiac and CNS defects (4).

Cytomeglovirus (CMV) is a largest virus of herpesviridae family. It is transmitted to infant during pregnancy, ingestion of infected human milk, direct contact with urine and saliva. Infants showed various complications such as microcephaly, intracranial calcifications, decrease in hearing and thrombocytopenic purpura etc. (5).

Herpes simplex virus-II (HSV-II) is a DNA virus of the herpesviridae family. *HSV-II* enters the infant through his or her eyes, skin, mouth and upper respiratory tract. Complications include meningo encephalitis, ulcers in cornea etc(6). It is useful to early diagnose of ToRCH group of agents because the risk of birth defects and fetal damage can be greatly reduced by the treatment of ToRCH group of agents. Direct method include polymerase chain reaction. Indirect method include Enzyme Linked Immuno sorbant assay and Chemiluminenescence linked immunoassay.

The main aim of this was study to find out the seropositivity of ToRCH infections in pregnant women who attended antenatal clinic (ANC) at tertiary care hospital, Udaipur, Rajasthan.

Materials and Methods

The present study was conducted in a tertiary care hospital, Udaipur, Rajasthan. Study was carried over one year. Total 111 non duplicate blood samples were collected. It was a prospective study. Ethical approval was obtained from the Human Research Ehtics Committee. Written and signed consent were obtained from the patients for voluntary participation in the study.

Inclusion criteria

Pregnant women of age group (18 to 45) attending antenatal clinic (ANC) of Obstetrics Gynecology Department of Tertiary care hospital, Udaipur were included in the study.

Exclusion criteria

Women having history of autoimmune disorder /immunocompromised status and taking any steriod therapy. Patients with medical and surgical ailments.

Collection of blood samples

About 4 to 5 ml of single sample for detection of Anti *Toxoplasma, Rubella, Cytomeglovirus and Herpes simplex virus-II* antibodies were collected aseptically by venepuncture using a sterile disposable needle in a vacutainer from pregnant women at the ANC by trained nursing staff under the supervision of the investigators.

Vacutainer used for collection and transport of the samples was labelled using a unique link number which was similar to the respective questionnarie.

Processing of blood samples

Blood sample collected were allowed to clot and centrifuged at 2500 to 3000 rotation per minute (rpm) for 5 to 10 minutes for serum seperation prior to testing. All sera samples were stored at 4 degree centrigrade until tested sera samples were tested for detection of Anti *Toxoplasma* IgM and IgG, Anti *Rubella* IgM and IgG, Anti *Cytomeglovirus* IgM and IgG and Anti *Herpes simplex virus-II* by Chemilumescence Immune Assay(CLIA) (Cobas e411 Hitachi) and *Herpes simplex virus-II* IgM by Enzyme linked immunosorbant assay (ELISA) (CAL BIOTECH).

Results and Discussion

A total number of 111 pregnant women were attending ANC in the tertairy care hospital. Blood samples were collected after filling the questionnaires from the agreed participating women (Table 1 and 2).

Table.1 Distribution of Socio-demographic and pregnancy related characteristic among the study participants (N=111)

(A) Distribution of study cases on the basis of age groups

Age	No. of cases	Percentage
≤ 20	10	09.01
21-30	68	61.26
31-40	27	24.32
≥40	06	05.41
Total	111	100

(B) Distribution of cases under study on the basis of past BOH

Obstetric history	No. of cases	Percentage
BOH	50	45.05
No BOH	61	54.95
Total	111	100

(C) Distribution of cases study on the basis of gestational age

Gestational age	No. of cases	Percentage
First trimester	65	58.56
Second trimester	25	22.52
Third trimester	21	18.92
Total	111	100

(D) Distribution of study cases on the basis of educational level

Educatinal level	No.of cases	Percentage
Illiterate	21	18.92
Primary	20	18.02
Secondary	45	40.54
Tertiary	25	22.52
Total	111	100

Table.2 Distribution pattern of ToRCH antibodies in seropositive study subjects

Pattern of antibodies	Toxoplasma (%)	Rubella (%)	CMV (%)	HSV-II (%)
Only IgG	2(1.80%)	23(20.72%)	30(27.02%)	1(0.90%)
Only IgM	1(0.90%)	15(13.51%)	20(18.01%)	1(0.90%)
Both IgG &IgM	0(00.00%)	5(4.50%)	2(1.80%)	0(00.00%)
Total	03(2.70%)	43(38.73%)	52(46.84%)	2(1.80%)

Pregnant women and immunocompromised patients are two major risk groups affected by the ToRCH group of agents. In pregnancy, the patients are not harmed; instead the foetus is affected, leading to the Bad obstetric history (BOH) such as abortion, stillbirth, miscarrige etc(7). Since maternal infections with ToRCH agents is initially asymptomatic and as the clinical diagnosis are unreliable, the diagnosis of these infection depends upon serological evidences.

In patient infected with the ToRCH group of agents, mainly two types of antibodies are produced against the infecting organisms i.e. immunoglobulin M (IgM) and immunoglobulin G (IgG). Detection of IgM and IgG antibodies are the sensitive indicators of acute and remote infections respectively.

In India, seroepidemiological (IgM) and studies of the women who were in the child bearing ages had shown that the maternal infection ranged for *Toxoplasma* was 7.3% to 29.01%, for *Rubella* 2% to 34.88%,for *CMV* 2% to 38.46% and *HSV-II* 2% to 13.44%.In the present study the serological diagnosis of the recent ToRCH agent infection were made on the basis of the presence of the specific IgM antibodies. Out of 111 screened pregnant women, Seropositvity (IgM/IgG) for *Toxoplasma* was 03(2.70%), *Rubella* 43 (38.74%), *CMV* 52(46.85%) and *HSV-II* was 02(1.80%).

In the current study, out of 111 screened pregnant women, *Toxoplasma* specific IgM and IgG antibodies were 02(1.80%) and 01(0.90%) respectively. Though,the lowest

reported *Toxoplama* specific IgM antibodies was 7.3% by Bhavesh et al.(8) and *Toxoplasma* specific IgG antibodies were reported 18%,29.01% and 42.01% by Bhavesh *et al.*, (8), Col Lavan *et al.*, (9) and Turbadkar *et al.*, (10) respectively. These differences can be explained on the basis of variation in cultural eating habits and socio-economic status among the different race groups and contact with pets.

In the present study, the presence of *Rubella* IgM antibodies were found in 15 (13.51%) of the pregnant women. In contrast higher seroprevalence were reported by M.R Sen et al.(11) 30.04% and lowest specific IgM was 2% which was reported by Bhavesh et al.(8)For *Rubella* specific IgG were found in our study was 23(20.72%). In contrast to present study 6% reported by Bhavesh *et al.*, (8) But *Rubella* is such a mild disease, that not more than 1 in 10 cases are recorded. The paradox lies in the fact that a large proportion of the cases are subclinical and that the clinical diagnosis is unreliable. Moreover, there is a considerable variation in the prevalence of the *Rubella* specific IgG antibodies among the women of the child bearing ages, with studies suggesting prevalence of 71.3% *Rubella* immunity, thus leaving about 1/3rd of the women susceptible to the Rubella infection. As the screening for the *Rubella* immunity was not done in this study, the serological diagnosis of the recent infections was made on the basis of the presence of the specific IgM antibodies. The history of the vaccination against *Rubella* could not be gathered from the study population, but on the basis of socio-economic status and the educational background, it could be presumed that most of them had not been previously vaccinated.

Cytomeglovirus is a member of the *herpes virus* family and it is found universally throughout all the geographical locations and in the area of low socio-economic conditions. For *CMV* specific antibodies IgM were found in 20(18.01%) in present study. Similar studies conducted by Col Lavan et al.(9) 99(13.58%). For CMV specific antibodies IgG were found in present study 30 (27.02%). Similar findings have been reported by Turbadkar *et al.*, (10) 233 (33.58%). In constrast to this lowest findings has been reported by Bhavesh *et al.*, (8) 16 (10.7%). This variation might be due to the difference in sensitivity of varoius kits used and the socio-economic state of the study population.

Neonatal Herpes which can be aquired in –utero from maternal infections, is quite severe and it is associated with a high morbidity and mortality (12). In the present study, *HSV-II* IgM evidence of infection was found in 01(0.90%). In contrast, a higher percentage reported by M.R.Sen *et al.*, (11) 151(33.5%); Bhavesh *et al.*, (8)11(7.3%).

In the present study, higher seropositivity for IgG and IgM were found in between 20 to 40 years age group for ToRCH agents. This is similar to the study conducted by M.R.Sen *et al.*, (11). In contrast few studies documented that prevalence increases as the age increases. This also highlights the need to continue to educate women of child –bearing age on prevention of ToRCH infections.

Maternal infection plays a critical role in pregnancy wastage and their occurance with BOH of complicated pregnancy is an significant factor. In present study the seroprevalence of Toxoplasma specific IgM and IgG among pregnant women with BOH was 1.36% and 2% respectively. Higher seropositvity for Toxoplasma IgM antibody was reported by Shashi *et al.*, (13) 42.5% in cases of BOH. Studies have proved that persistence of encysted form of toxoplasma in chronically infected uteri and their rupture during placentation leads to infection of baby

in the first trimester and often recurrent miscarriages.

In present study, the seroprevalence for Rubella IgM and IgG in pregnant women with BOH were both 7(14%). Namrata *et al.,* (14) reported a lower incidence of 6.5% whereas Turbadkar *et al.,* (10) reported a higher incidence of 26.8 in cases of BOH. The variation in Rubella seropositivity lies in the fact that a large proportion of the cases are subclinical and the clinical diagnosis are unreliable. Moreover, in recent infection serological diagnosis was made on the basis of presence of Rubella specific IgM antibodies; whereas the prevalence of Rubella specific IgG antibody was not correlated with the history of vaccination of the study population in most of the study carried out.

In present study specific IgM antibody was found 07(14%) for CMV in screened pregnant women with BOH. Turbadkar *et al.,* (10) reported 8.4% for specific IgM antibody in pregnant women with BOH which is similar to the present study. In constrast, M.R.Sen *et al.,* (11), Shashi *et al.,* (13) reported higher incidences of 34.7%, 29.4% respectively. For specific IgG antibody CMV was found in present study was 11(22%) in pregnant women with BOH. In contrast to this Ghazi *et al.,* (7) found higher incidence of 92.1% for specific antibody. The role of CMV infection is not very significant in the miscarriage cases because most of the general population is immune to this pathogen as many studies have documented the IgG antibodies levels varying from 87% to 97%.

Herpes family of viruses is known to cause latent infection, there may be reactivation of virus during pregnancy and it is acquried mainly during passage through birth canal. In present study, seropositivity for specific HSV-II IgG was 01(2%) among screened pregnant women with BOH which is similar to our findings Turbadkar *et al.,* (10) reported 3.3%.Primary infection with HSV-II accounts for half of the morbidity and mortality in neonates, while other half results from reactivation of an old infection.

Harmful results can occur if maternal infection with *Toxoplasama gondii* occurs during first trimester of pregnancy leading to the parasite entering the foetal circulation through placenta. This may result in severe congenital toxoplamosis and result in death of the foetus and also result in spontaneous abortion. In the present study, higher seropositvity for Toxoplasma specific IgM was found comparatively higher in first trimester 01 (1.53%) of pregnancy. This is similar to the study conducted by M.R.Sen *et al.,* (11) who also reported higher Toxoplasma IgM seroposivity in first trimester.

In case of of specific IgM and IgG for CMV were 13.84% and 32.30% respectively in first trimester of pregnancy. Same observation was shown by M.R.Sen *et al.,* (11).

The risk of the congenital Rubella infection following a maternal infection ranged from 5% to 50% in various studies, with an increasing severity when it was acquried in the first trimester of the pregnancy. This is similar to our findings Rubella specific IgM 13(20%), IgG 18(27.69%) antibodies occuring in the first trimester of pregnancy.

The primary HSV-II infection during pregnancy is responsible for half of the mortality among neonates. M.R.Sen *et al.,* (11) higher infection of HSV-II occurs in first trimester of pregnancy which is similar to the present study.

In the present study, higher IgM and IgG seropositivity for ToRCH group of agents were found in women with lower level of

education (15}. It is matching with the study carried by A.M Deji *et al.*, (16) who also reported higher seropositivity in lower education group. Thus, indicating that lower level of education are associated at increased risk of ToRCH infection. So, there is need to educate the women of child bearing age about the prevention of ToRCH group of agents and to incorporate awareness programme and screening of ToRCH infection during antenatal period.

In conclusion it is recommended that all pregnant women with BOH should be routinely screened for ToRCH agents at the first antenatal visit as ToRCH infections play a major role in adverse fetal outcome. Awareness amongst clinicians and patients about ToRCH infections and their consequences should developed and prophylactic measures should be adopted in vulnerable patients specially with BOH.

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