DOI: NJLM/2015/12994:2061

Original Article

Microbiology Section

Assessment of Utility of Serological Test Against TORCH Group of Agents for Bad Obstetric Outcome in Tertiary Care Hospital

HASSANI UMESH, SOMALWAR SAVITA, THOMBARE VILAS

ABSTRACT

Introduction: Poor pregnancy outcome is multi factorial. Maternal infections have been considered as one of the significant factors in causation of poor pregnancy outcome elsewhere but it has not assumed much significance in India, as data is scanty because of the technical difficulties in isolating the organisms and the requirement for use of commercial diagnostic kits which are expensive. This study is a case control study to study the utility of testing for TORCH IgM antibodies in patients having a bad obstetric history.

Aim: Present study was conducted among pregnant women with bad obstetric history, coming to ANC clinic of our hospital to shed some light on association between seroprevalence & recent infection with *Toxoplasma*, *Rubella virus*, *Human Cytomegalovirus* (HCMV), and *Herpes Simplex virus* (HSV), by the detection of IgM Antibodies.

Materials and Methods: Thirty seven women in the age range from 18-35 years with bad obstetric outcome were included in this study after clearance from Institutional Ethical Committee. Thirty five healthy women with previous normal obstetric outcome were also included as age match control group. Serum samples from aseptically collected blood from the enrolled cases after informed consent was preserved in 0.5 ml aliquots at -20°C till tested. All sera were tested for the identification of class specific IgM antibodies by ELISA for *Toxoplasma*, *Rubella virus*, HSV I and II and CMV using IgM capture ELISA kit according to manufacturer's instructions. Data was maintained in Microsoft Excel and tests of proportions and Pearson's chi test for significance were employed using Epi info.

Results: In the study group (group I), 10 (27.02%) women were positive for IgM antibodies against *Toxoplasma*, *Rubella*, *Cytomegalovirus*, *Herpes* I and II either alone or in combination. In the Control group (group II), IgM antibodies were detected in 5(14.28%) cases against *Cytomegalovirus* and *Herpes* I and II each. One case had antibodies against *Rubella*. When compared with the control group only *Toxoplasma* infection amongst TORCH agent with p value at one degree of freedom is statistically significant for bad obstetric outcome.

Conclusion: In Present study detection of IgM Antibodies has been performed by capture ELISA which reflects recent infection whereas most of the other studies have relied on IgG antibodies. Serological diagnosis may not be reliable indicator of maternal infection, hence it can be used as screening test in cases were there is strong suspicion of maternal infection and should be confirmed by tests with higher specificity.

Keywords: Capture ELISA, Congenital anomalies, IgM antibodies, *Toxoplasma Gondii*

INTRODUCTION

Fetal death, premature birth, intrauterine growth retardation or persistent postnatal infection with subsequent developmental malformations may result from In utero infections (1). *Toxoplasma gondii*, *Rubella virus*, *Human Cytomegalovirus* (HCMV), and *Herpes Simplex virus* (HSV), the so called TORCH agents are the most common agents implicated in congenital infections [2].

Nahmias et al., [3] in 1971 introduced the acronym "TORCH" to highlight a group of agents which affect the foetus and newborn, namely *Toxoplasma gondii*, *rubella* virus, *cytomegalovirus* (CMV), and *herpes simplex virus* (HSV).

Apart from pre-clinical pregnancy loss which is 22-30 %. It is well realized that at least 12-15% of all recognized conceptions end in miscarriage [4].

Maternal infections have been considered as one of the significant factors in causation of poor pregnancy outcome but data is scanty because of the technical difficulties in isolating the organisms and the requirement for use of commercial diagnostic kits which are expensive [5].

At present, there is no hard evidence that bacterial or viral infections can cause recurrent abortions. There are also reports saying that most of the infections of the mother will not result in fetal infection [4].

These maternal infections are difficult to diagnose on clinical grounds as they are initially unapparent or asymptomatic [6]. The prevalence of these infections also varies from one geographical area to another [7].

AIM

We conducted the study among pregnant women with bad obstetric history coming to Ante Natal Clinic of our hospital to shed some light on association between seroprevalence and recent infection with *Toxoplasma*, *Rubella virus*, *Human Cytomegalovirus* (HCMV), and *Herpes Simplex virus* (HSV), by the detection of IgM Antibodies.

MATERIALS AND METHODS

After Institutional Ethical clearance, 37 women (Group I) in the age range from 18-35 years attending ANC clinic of Obstetric and Gynecology Department of NKP Salve Institute of Medical Sciences Nagpur ,were enrolled on the basis of recent history of abortion/s, preterm labor, intrauterine fetal death (IUFD), stillbirth, or congenital anomalies between November 2010 – January 2012. Age match control group of 35 healthy women (Group II) with previous normal obstetric history was also inducted in the study. Written informed consent was obtained from the enrolled patients and history was collected as per the Performa which considered age, gravid, parity, previous miscarriages, pregnancy induced hypertension, diabetes and infection with hepatitis/HIV virus.

3 ml blood samples was collected aseptically from patients and controls, centrifuged for 10 min x 3000 rpm, then sera were separated and divided into 0.5ml aliquots and kept at -20°C till tested. All sera were tested for the identification of class specific IgM antibodies by ELISA for *Toxoplasma*, *Rubella* virus, HSV I and II and CMV using IgM capture ELISA kit (Asia Lion Biotech).The tests were done as per the directions given in the manual supplied along with the kits. The results were read at 450 nm in the ELISA reader (Merck) and interpreted as follows-

Cut off: 0.10+ average value of negative control

Positive: OD value equal to or greater than the cut off

Negative: OD value less than the cut off.

Syphilis was diagnosed using RPR test. Tests for presence of HIV and HBsAg were done by immunochromatographic ELISA (Rapid test) and results were recorded in the Performa.

Statistical Analysis: Data was maintained in Microsoft Excel 2010 and test of proportions and significance were carried out using Epi-info software.

RESULTS

The study group of bad obstetric history (Group I) consists of abortions 21(56.75%), Intrauterine death 6 (16.21%), Premature labor 3 (8.10%), Congenital anomalies 4 (10.81%) and Intra Uterine Growth Retardation 3 (8.10%).

The study group, 10 (27.02%) women were positive for IgM antibodies against *Toxoplasma*, *Rubella*, *Cytomegalovirus*, *Herpes* I and II either alone or in combination. In the Control group, IgM antibodies were detected in 5 (14.28%) cases against *Cytomegalovirus* and *Herpes* I and II each. One case had antibodies against *Rubella*. When compared with control group, *toxoplasma* infection, amongst TORCH group of agents with p-value at one degree of freedom is statistically significant for bad obstetric outcome [Table/Fig-1].

IgM Ab against ToRCH group of agents was not seen in patients with outcome of congenital anomalies and IUGR. The seropositivity of IgM antibodies against *Toxoplasma*, *Rubella*, CMV and HSV I and II against cases of abortions, Intrauterine deaths and premature deaths are shown in [Table/Fig-2].

DISCUSSION

The present study shows that the value of IgM TORCH agent levels in pregnant females are almost similar in cases having bad obstetric history, compared to normal population. Various reports have suggested that specific infectious agents cause recurrent abortions, but there is no concrete evidence as to role of specific bacterial or viral agents in recurrent abortions [4].

Summers (1994) suggested that recurrent miscarriage due to infection occurs with frequency that is much low, infection is occasional cause of sporadic abortion and consistent with statistical probability [8].

In prospective studies, evidence linking infection and recurrent pregnancy loss could not be reproduced, hence limited evidence linking infection and recurrent pregnancy loss in humans remains largely anecdotal.

TORCH agent	Seropositivity Group I BOH (n=37)		Seropositivity Group II Controls (n=35)		p-value
	No.	Percent	No.	Percent	
Toxoplasma	3	8.10%	0	0%	0.04
Rubella	1	2.7%	1	2.85%	0.48
Cytomegalovirus	4	10.8%	5	14.28%	0.33
Herpes simplex virus	2	5.40%	5	14.28%	0.11
Total	10	27.02%	11	31.42%	0.36

[Table/Fig-1]: The seropositivity of TORCH agents
*One case showed mixed infection with *toxoplasma* and Cytomegalo virus

Bad obstetric history	*Toxoplasma* IgM +ve		*Rubella* IgM +ve		*Cytomegalovirus* IgM +ve		*Herpes* simplex I and II IgM +ve	
	No.	Percent	No.	Percent	No.	Percent	No.	Percent
Abortions n=21 (56.75%)	2	5.40	0	0.00	1	2.70	1	2.70
Intrauterine death n=6 (16.21%)	1	2.70	1	2.70	1	2.70	0	0.00
Premature labour n=3 (8.10%)	0	0.00	0	0.00	2	5.40	1	2.70

[Table/Fig-2]: TORCH agents with different presentation of BOH cases

Toxoplasma: In the present study seropositivity for *toxoplasma* was seen in 3 (8.10%) cases in the group I whereas all the pregnant females in group II were seronegative.

Many observers in various studies conducted in different parts of the world have detected *Toxoplasma* IgM antibodies among pregnant women using ELISA, which ranges from 13% to 43%. Yashodhara et al., in a study conducted in southern India reported the toxoplama IgM in 13.1% cases [5]. Similar study conducted by Surpam et al., in Central India reported toxoplama IgM in 14.66 % cases [9].

CMV: Among the study group cases the seropositivity for CMV IgM antibodies was 4 (10.8%) cases whereas in control group was 5 (14.28%) cases.

Role of CMV infection is not very significant in the miscarriage cases. Most of the general population is immune to this pathogen as many studies have documented the IgG levels varying from 87-97%. Rhodier et al., [10] 1995 in his study found the IgG levels as high as 97.2%,Ghazi et al., [11] 2002 reported levels of 92.1% The IgG levels of CMV antibodies in study conducted by Ustacelebi et al., [12] 1986 reported levels of 87.8% .

Yashodhara et al., [5] in a prospective study conducted in southern India reported that 5.8% cases were positive for CMV IgM, but there was no association with adverse outcome of pregnancy.

Rubella*:* In the present study the seroprevalence of *Rubella* IgM antibody was 2.7% in the study group, which was almost similar to that found in the control group. Across the globe there is a considerable variation in the prevalence of *rubella* antibodies among women of child bearing age. Various studies conducted in India have reported figures 53 to 94.1 % [13-15]. A survey in Hong Kong showed that 90% nulliparous and 93% multiparous women of child bearing age were positive for *rubella* antibodies [16]. Similarly in a seroepidemological study of *rubella* in Saudi Arabia, El Mekki and Zakki [17] found a seropositivity rate of 94 % in women of child bearing age group. Padmaja et al., [18] in seroprevalence of immunity to rubella in pregnant women studied seroprevalence in 283 pregnant women, (65.7%) were IgG-positive and thus had immunity against *rubella* and 13 women (3%) were IgM positive. HSV: IgM antibodies against HSV I and II were detected in 5 (14.28%) cases in control group whereas it was detected in only 2 (5.40%) cases in control group.

Herpes family of viruses is known to cause latent infection, there may be reactivation of virus during pregnancy and it is acquired mainly during the passage through birth canal. Primary infection with HSV II accounts for half of the morbidity and mortality in neonates [19].

HSV infection in asymptomatic women with recurrent infection during pregnancy was reported to be 0.6-3% in previous study [20].

RPR test and immunochromatographic tests for presence of HIV Ab and HBs Ag were negative in all the females in group I as well as group II.

LIMITATION

As this study was performed with limited number of patients a larger trial will be needed to conclusively link the serological diagnosis with maternal infections.

CONCLUSION

In present study detection of IgM Antibodies has been performed by capture ELISA which reflects recent infection whereas most of the other studies have relied on IgG antibodies. Serological diagnosis may not be reliable indicator of maternal infection, hence it can be used as screening test in cases were there is strong suspicion of maternal infection and should be confirmed by tests with higher specificity.

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AUTHOR(S):
1. Dr. Hassani Umesh
2. Dr. Somalwar Savita
3. Dr. Thombare Vilas

PARTICULARS OF CONTRIBUTORS:
1. Lecturer, Department of Microbiology, NKP Salve Institute of Medical Sciences, Nagpur, India.
2. Lecturer, Department of Microbiology, NKP Salve Institute of Medical Sciences, Nagpur, India.
3. Professor and Head of Department, Department of Microbiology, NKP Salve Institute of Medical Sciences, Nagpur, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Hassani Umesh,
Lecturer, Department of Microbiology, NKP Salve Institute of Medical Sciences, Nagpur, India.
E-mail: drumeshhassani@yahoo.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Publishing: **Oct 01, 2015**