

## Adverse reproductive outcome induced by Parvovirus B19 and TORCH infections in women with high-risk pregnancy

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### Abstract

**Introduction:** The frequency of fetopathogenic viruses and *Toxoplasma gondii* infections in the TORCH group (*Toxoplasma gondii*, rubella virus, cytomegalovirus (CMV), herpes simplex virus) together with Parvovirus B19 (B19) in pregnant women with bad obstetric history (BOH) and/or concurrent pregnancy complications was investigated.

**Methodology:** Sixty women (20-35 years) with BOH and/or antecedent pregnancy complications were studied. Twenty-nine healthy pregnant women matched for age, parity and gestational age served as controls. Sera were analyzed for IgM antibodies for B19 and TORCH agents by ELISA. Cord blood and 33 placental tissues from six malformed newborns were tested for B19 DNA by PCR.

**Results:** Out of 60 high-risk pregnant women, 47 (78%) had BOH while 23 (38.3%) had underlying complications including polyhydramnios (n=10), oligohydramnios (n=6) and intrauterine growth restriction (n=7). Adverse outcomes occurred in 36 (60%) high-risk cases. All 16 cases with polyhydramnios/oligohydramnios resulted in preterm stillbirths while the remaining 20 cases resulted in seven abortions, six newborns with congenital malformations, four full-term stillbirths and three cases of non-immune hydrops fetalis (NIHF). IgM positivity to *T. gondii*, rubella, cytomegalovirus, herpes simplex virus and B19 virus was 8.3%, 15%, 30%, 3.3% and 13.6% respectively. B19 infection caused NIHF in three cases and cardiac anomaly in one. All placental tissues and cord blood were negative for B19 DNA. None of the controls had IgM antibodies to any pathogen.

**Conclusions:** Women with BOH and/or pregnancy complications had a high frequency of TORCH and parvovirus B19 infections causing fetal wastage, IUGR, NIHF and congenital malformations.

**Key words:** abortion; BOH; congenital malformation; hydrops fetalis; Parvovirus B19; pregnancy complications; TORCH

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### Introduction

Recurrent pregnancy wastage due to maternal infections transmissible *in utero* can be caused by a wide array of pathogenic organisms mostly belonging to the TORCH group (*Toxoplasma gondii*, rubella, cytomegalovirus [CMV], Herpes simplex viruses [HSV-2]), *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and recently parvovirus B19 (B19) [1-3]. Infection with these agents can result in significant morbidity and mortality especially in developing countries [3-5]. All these pathogens infect and affect the fetus at various stages of gestation and can cause various types of feto-maternal complications either once, as is usual in rubella virus infection, or recurrently, as seen notably with *T. gondii*, CMV, *Chlamydia trachomatis* and probably B19 virus [1-3]. In addition, studies have found that parvovirus B19 has a 33% vertical transmission rate in pregnant women [6.] We have previously reported

a frequency of 19.8% for B19 IgM in a study on 116 women with recurrent spontaneous abortions [7]. Most published reports are retrospective studies in women with bad obstetric history (BOH) to find the possible etiological agent, usually with individual pathogens causing abortion, stillbirth etc. There are no reports globally which have studied women with history of BOH and who were currently pregnant with high-risk factors such as polyhydramnios/oligohydramnios and where outcome of such pregnancies were studied. Hence a prospective study was designed to find the infections due to TORCH agents and parvovirus B19 in pregnant women with BOH and the clinical outcome in such cases. The investigation also tested the congenitally malformed newborns of such mothers due to these pathogens.

## Methodology

### *Study setting, exclusion criteria and blood samples*

A prospective study was designed that included 89 pregnant women, preferably belonging to poor socio-economic strata, in the age group of 20-35 years, attending the antenatal clinic at Chhatrapati Shahuji Maharaj (CSM) University, Lucknow between September 2005 to August 2006. The study group comprised of 60 high-risk pregnant women who had previous bad obstetric history and/or some current pregnancy complication such as polyhydramnios, oligohydramnios or intrauterine growth restriction (IUGR). Additionally, 29 healthy pregnant women with no history of recent pregnancy complications and matched for age, parity, and gestational age were included as controls.

Exclusion criteria were presence of hypertension, diabetes mellitus, eclampsia of pregnancy, and Rh incompatibility. Detailed clinical history, physical examination, and conventional laboratory investigations were conducted. A preformatted questionnaire, including the socio-economic status, was completed during the antenatal follow-up period from gestation to birth. Delivery outcome was recorded for cases with reference to the gestational age and mode of delivery. Information on stillbirths and congenital malformations was recorded.

Blood samples (5 ml) were drawn aseptically from all 89 enrolled cases and controls for routine investigations. An aliquot of sera was preserved at -70°C for study. In addition, at the time of delivery, placental tissue (post-delivery discarded placenta when available) of 33 cases was taken and cord blood of six children who had congenital malformations was collected. All serum samples were sent for sero-molecular analysis for parvovirus B19 infections at the Virology unit, Department of Microbiology, Sanjay Gandhi Post-Graduate Institute of Medical Sciences, Lucknow. IgM antibodies for TORCH infections were tested by commercial ELISA kits (Biomérieux, the Netherlands and Bio-Rad, Hercules, CA, USA) using a set of positive and negative controls in each assay. An in-house ELISA test was performed for anti-B19 IgM antibodies as described previously [8]. Optical density (OD) values were recorded and an index value of more than or 1.1 in comparison to the OD of the cut-off control was taken as positive.

### *DNA extraction and PCR for parvovirus B19*

B19 DNA was extracted from 200 µl of each serum specimen after digestion with 20 µl proteinase

K following the procedures outlined in the QIAamp Ultrasens virus kit (Qiagen, GmbH, Hilden, Germany). Placental tissue DNA was extracted by QIA amp Mini kit (tissue protocol). B19 DNA was then amplified by nested-PCR and was performed in a Cleanspot PCR Workstation (Coy Laboratory Products, Grass Lake, Michigan, USA; WHO) using a set of primers from VP1 unique regions of B19 namely, primer B3 – TGTGTGTTGTGTGCAAC - (nt 2193-2209) and primer B4 – CAAACTTCCTTGAAAATG – (nt 3119-3235). One microliter of PCR product was then subjected to a second round of amplification using internal primers B5 – CAAAAGCATGTGGAGTGAGG - (nt2229-2245) and B6-GTGCTGTCAGTAACCTGTAC - (nt 3065-3082). Measures to prevent contamination in PCR reactions as recommended were carefully observed. On agarose gel electrophoresis and ethidium bromide staining PCR amplicons (35 cycles, Perkin Elmer, GeneAmp 9600, USA) were 942 bp in the first round and 853 bp after the second round of amplification respectively. These amplicons were regarded as evidence of B19 DNA positivity. Sterile distilled water was used as the negative control while cloned and purified B19 DNA (from a case of erythema infectiosum) was used as a positive control (donated by Dr Y Matsunaga, NIID, Tokyo, Japan) [9].

### *Statistical analysis*

Data was analysed using the  $\chi^2$  test, Fisher's exact or "t" test. A p value of < 0.05 was considered statistically significant.

## Results

The history of 60 high-risk pregnant women revealed that 47 (78 %) had BOH which included past abortions in 28 cases, IUGR in 15 cases, and stillbirths in four cases. On examination and investigations, antecedent pregnancy complications detected were polyhydramnios (n = 10), oligohydramnios (n = 6) and IGUR (n = 7) in 23 (38.3%) cases. Socio-economically 30 cases belonged to a low-income group while 26 and 4 cases belonged to middle- and high-income groups respectively. Thus both low- and middle-income groups were predominant over the high-income group (p < 0.01). Most of the cases were asymptomatic (75%) while among the symptomatic cases common clinical features observed were fever, anemia, facial rash, and fatigue. The comparison of the frequency of these clinically apparent cases with asymptomatic

**Table 1.** Parvovirus B19 and TORCH infection in pregnant women with BOH/current pregnancy complication

Type of pathogen	IgM antibodies by ELISA Number of positive	Percentage positive
<i>Toxoplasma gondii</i>	5/60	8.3%
Rubella	9/60	15.0%
CMV	18/60	30.0%
HSV-2	2/60	3.3%
Parvovirus B19	6/60	13.6%

CMV = cytomegalovirus; HSV-2 = herpes simplex virus

cases was statistically significant ( $p < 0.001$ ). Infection with any one of the TORCH agents or parvovirus B19 was detected in 24 of the 60 (40%) cases based on the detection of agent specific IgM antibodies. Antibody positivity to *T. gondii*, rubella, CMV, HSV-2 and B19 virus was 8.3%, 15%, 30%, 3.3% and 13.6% respectively (Table 1). None of the 29 healthy pregnant women in the control group was positive for IgM antibody to any of the viral infections and this difference is statistically significant ( $p < 0.001$ ).

Post-delivery adverse outcomes were observed in 36 of the 60 (60.0%) cases which included stillbirths ( $n = 20$ ), abortions ( $n = 7$ ), congenital malformations ( $n = 6$ ), and non-immune hydrops fetalis (NIHF) ( $n = 3$ ). The association of IgM antibodies to parvovirus B19 and various TORCH infections in the study group is shown in Table 2. Antibody positivity was highest for CMV (30%) followed by rubella virus (15%) and B19 (13.6%). In five cases of oligohydramnios and six cases of IUGR no IgM antibodies to the above agents were detected.

Cord blood samples in all three cases of NIHF were positive for IgM antibodies to parvovirus B19. Out of six cases of congenital malformations, one case of cardiac anomaly was detected which had antibodies to more than one pathogen including parvovirus B19 (Table 3). One case of blighted ovum was negative for IgM antibodies to all pathogens tested but had inevitable abortion and hence dilatation and curettage had to be performed.

## Discussion

Infections play a critical role in pregnancy wastage and their occurrence in patients with BOH or complicated pregnancy is a significant risk factor [1,2,4,5]. All viral pathogens usually cause a primary

maternal viremia which may infect the placenta and thereby the fetus with the exception of HSV-I or II, which causes an ascending infection via the genital tract to fetal membranes and then to the fetus [9,10]. Detection of agent specific IgM antibodies in a single serum sample have been taken as a reliable indicator of recent infection and hence this method was employed here. In the present study *T. gondii* infection was found in 8.3% of pregnant women in the study group. In contrast, Surpam *et al.* reported overall IgM antibody positivity of 14.6% in 150 cases of BOH but it rose to 27.2% in such women who had two or three abortions [11]. Similarly, in India, Turbadkar D *et al.* found toxoplasma IgM and IgG antibodies in 10.5% and 42.1 % cases of BOH [12]. Studies have proved that persistence of encysted forms of toxoplasma in chronically infected uteri, and their rupture during placentation, lead to infection of the baby in the first trimester and often to recurrent miscarriages [9-13].

About 35% to 40% of women in the reproductive age group in our country are known to be susceptible to primary rubella infection which can result in hypoplastic organomegaly as a consequence of slow multiplication of infected fetal cells. However, clinical features such as delayed milestones and behavioral/psychosomatic disorders may not be evident at birth [13,14]. In our study, positivity for IgM rubella antibodies was 15% in the study group compared to the controls ( $p < 0.01$ ). All four cases of congenital malformation and one case of NIHF were infected with rubella virus. Other researchers have reported seropositivity to rubella infection ranging from 4 to 17.7% [3,5,9,13,14,15].

The need for serological evaluation of CMV specific IgM during pregnancy has been supported by various investigators [16,17]. The present study showed a positivity rate of 30% for CMV specific

**Table 2.** Association between IgM antibody to parvovirus B19 and TORCH agents in pregnant women with BOH and/or current pregnancy complication (n = 60) and their clinical outcomes

Clinical outcome of pregnancy	Toxoplasma No. (%)	Rubella No. (%)	CMV No. (%)	HSVII No. (%)	ParvovirusB 19 No. (%)
Abortions (n = 7)	3 (42.8)	2 (33.3)	4 (57.1)	1 (14.2)	1 (14.2)
Congenital malformations (n= 6)	0 (0)	2 (33.3)	3 (50.0)	1 (16.6)	1 (16.6)
stillbirth (n= 20) (preterm = 16, full term = 4)	1 (5)	1 (5)	7 (35)	0 (0)	0 (0)
NIHF (n = 3)	0 (0)	1 (33.3)	1 (33.3)	0 (0)	3 (100)
Total = 36	4 (6.6)	6 (10)	15 (25)	2 (3.3)	5 (8.3)

CMV = cytomegalovirus; HSV-2 = herpes simplex virus; NIHF = non-immune hydrops fetalis

**Table 3.** Cases of congenital malformations positive for TORCH and Parvovirus B19

Congenital anomaly	Number of newborns	Type of infection
Hydrocephalus (one case with spinal defect)	3	Toxoplasma, Rubella and CMV
Meningomyelocele	1	Rubella and CMV
Anencephaly	1	Rubella and CMV
Cardiomegaly	1	Rubella, CMV and Parvovirus B19

CMV = cytomegalovirus

IgM antibodies which was commonly seen in cases of abortions, unexplained still-births, and congenital malformations.

Primary infection with HSV-2 acquired by women during pregnancy accounts for half of the morbidity and mortality from HSV-2 among neonates while the other half results from reactivation of an old infection [1,2,9,10,11]. The seropositivity rate of IgM HSV-2 in our study group was 3.3%. In other previous reports, HSV infection ranged from 0.6 to 3% in asymptomatic women with recurrent infection during pregnancy [10,11].

Human parvovirus B19 infection has been detected in a number of developed countries throughout the world with adult seroprevalence rates varying from 30% to 60% in the adult population [4,6,7].

Fetal effects of B19 infection during pregnancy can result in fetal anaemia, spontaneous abortion, congenital anomalies, and NIHF [6,18-22]. The interval between maternal infection and the occurrence of foetal hydrops is often four to five weeks but may be as long as 11 weeks [21]. We detected three cases of NIHF who had recent parvovirus B19 infection.

In the present study, a very high percentage (60%) of adverse effects was observed, which may be related to the fact that the majority of the patients were from rural areas and were specifically chosen because they belonged to a socio-economically poor demographic and had a concurrent pregnancy complication, especially polyhydramnios /oligohydramnios. It may be noted that Chhatrapati Shahuji Maharaj Medical University, Lucknow, is a

free non-referral government hospital and that about 40% of the general population of India are known to be below the poverty line. Since the majority of people living in developing countries are usually socio-economically poor we specifically targeted women in this group for our study. Adverse outcomes occurred in 36 (60%) of the pregnancies. Infectious etiology in the rest of the cases included chlamydial and mycoplasmal infections while non-infectious etiologies such as genetic factors (*e.g.*, aneuploidy), chromosomal abnormalities, uterine anomalies (*e.g.*, leiomyoma, polyps), immunologic causes (*e.g.*, anti-phospholipid syndrome), environmental factors, host factors and idiopathic factors [23] were also possible. The prenatal diagnosis and prognosis of oligohydramnios has been discussed previously [23]. We also detected three cases of NIHF and all were anti-B19 IgM positive while one case had multiple viral infections (IgM antibodies to CMV and rubella) in addition to B19. NIHF due to B19 virus has been reported only occasionally and reports of congenital malformation due to B19 infection are rare [22]. However, B19 DNA was not detected in placental tissues, which could be due to PCR inhibitors such as hemoglobin; furthermore, tissue distribution of B19 in infected tissues is not known. Direct detection of viral particles or genomes in maternal blood is of little help since viremia seldom persists for more than two weeks in an immunocompetent individual [20,21]. Although detection of B19 DNA in maternal blood has the best diagnostic sensitivity for identifying maternal infection, supplementary measurement of VP1 IgG avidity and VP2 IgG epitope-type specificity (ETS) improves the precision of diagnosis and management of pregnant women affected by B19 virus [21]. PCR performed on amniotic fluid has sensitivity greater than 97% and specificity of 79-99% [18]. Fetal cord blood sampling is associated with a 1% fetal loss rate and IgM antibodies do not appear in the fetal circulation until after 22 weeks of gestation [6].

In conclusion, we recommend that all high-risk pregnant women, even if asymptomatic, should be screened for TORCH and parvovirus B19 infections. Early diagnosis and appropriate intervention will help in proper management of these cases.

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