Original Article

Risk of vertical transmission of SARS CoV-2 infection to neonates born to covid positive mothers: A pilot study from a tertiary care hospital of North India

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ABSTRACT

Objectives: To assess the risk of neonatal SARS-CoV-2 infection born to the women with confirmed SARS-CoV-2 infection.

Materials and methods: This prospective study was conducted at single tertiary hospital from September 2020 and May 2021. 50 pregnant women with confirmed SARS-CoV-2 infection and 50 neonates were included for analysis. We performed comprehensive testing of all biological samples for vertical transmission including the cord blood immunoglobulin.

Results: We detected SARS-CoV-2 in one fetal membrane and one amniotic fluid sample. We also demonstrated presence of anti-SARS-CoV-2 IgM antibodies in cord blood of 3 neonates. Though none of the samples of vaginal secretion, breast milk and nasopharyngeal swab from neonates were tested positive for covid infection via RT-PCR. We demonstrated presence of anti-SARS-CoV-2 IgG antibodies in the cord blood which had shown positive correlation with increasing disease to delivery interval and disease severity.

Conclusion: Vertical transmission of SARS-CoV-2 is possible. As virus was not detected in cervicovaginal secretions and breast milk so vertical transmission through this mechanism seems unlikely. Presence of IgG in cord blood is suggestive of passive immunity acquired from mother. This finding has greater clinical implication as large number of expecting mothers are being vaccinated.

1. Introduction

In last two decades, the world has witnessed many serious viral epidemics like SARS CoV in 2002–03, H1N1 influenza in 2009, MERS in 2012–13 and SARS-CoV-2 in 2019. In March 2020, covid-19 outbreak has been declared a pandemic by WHO. SARS COVID-19 is mainly transmitted through droplets, but other transmission routes have also been hypothesized. Transplacental transmission of SARS-CoV-2 has raised contradictory scientific evidences from various studies. Few cases of perinatal transmission are described in the literature but it remains unclear whether the spread has occurred through transplacental, transcervical route or environmental exposure [1–4]. The study conducted by Li et al. [5] revealed that the angiotensin converting enzyme (ACE2) receptor used by SARS-CoV-2 was widely distributed in specific cell types of the maternal-fetal interface cells and fetal organs. SARS-CoV-2 infect the host cells by recognizing human receptor ACE2 on it. So there is concern of vertical transmission due to tissue tropism. Presence of viral RNA in 15% of infected patients raise the concern of transmitting the virus through blood [3]. In addition, severe hypoxemia associated with SARS-CoV-2 infection can cause damage to placental barrier and could potentially lead to vertical transmission and intra-uterine infection.

Serology has played important role in diagnosis of few congenital infections like toxoplasmosis and syphilis, however its role in diagnosis of SARS-CoV-2 is not well established as yet. The possibility of a vertical transmission of the infection would have huge clinical and scientific implications. Although only few studies have confirmed the *trans-placental* route of transmission of SARS-CoV-2, this phenomenon needs further clinical investigation. This study aimed to assess the risk of neonatal SARS-CoV-2 infection born to the women with confirmed SARS-CoV-2 infection.

2. Materials and methods

This prospective study was conducted in the department of obstetrics and gynecology at AIIMS Patna, after obtaining clearance from the institute ethical committee (AIIMS/Pat/IEC/2020/573). A total of 76 pregnant women with COVID-19 were admitted for delivery between September 2020 and May 2021. 50 pregnant women with 50 newborns were included for final data analysis. All pregnant women had disease confirmation through nasopharyngeal RT-PCR. All data pertaining to maternal characteristics (age, parity, significant past medical history), onset and duration of symptoms, pregnancy information (gestational age at diagnosis, number of fetuses, pregnancy related complications, radiology findings, laboratory findings, ventilation support, ICU admission, estimated fetal weight, fetal abnormalities), delivery information (gestational age at delivery, delivery method, intrapartum complications, postpartum complications, placental pathology) and neonatal outcomes (evidence of COVID-19, NICU admission, respiratory morbidity, duration and type of ventilation support, infectious morbidity, neurological morbidity) were recorded. Disease severity and treatment institution were as per then national guideline at the time of admission [6].

Sample collection: To validate the pregnant woman's SARS-CoV-2 status, nasopharyngeal and oropharyngeal samples were collected for RT-PCR at the time of admission. Vaginal swab was obtained from posterior fornix before induction of labour/cesarean operation. In vaginal delivery, a sterile needle inserted through the vagina, and 5 ml amniotic fluid was carefully taken into the syringe in sterile conditions just before the rupture of the membranes. Similarly, in case of cesarean section, 5 ml of amniotic fluid was obtained in sterile conditions after giving uterine incision but before the rupture of the membranes. Placental swab was taken from the fetal surface after washing the placental tissue thoroughly with normal saline to remove any maternal blood contaminants. Fetal membrane sample was obtained after separating the two layers of amnion and chorion. Breast milk was obtained using sterile technique within 48 h of birth. Samples were placed into sterile tubes containing 3 mL of viral transport media (VTM) and transferred to virology lab within 30 min.

5 ml of cord blood was collected from the cord in plain vial and sent for IgM and IgG analysis using SCoV-2 Detect™ IgM and IgG ELISA kits which is a qualitative immunoassay (InBioS). Placental tissue was fixed in 10% formalin and sent to pathology department for histological evaluation. Sections underwent routine processing, embedding, sectioning at 5 μm and staining with H&E. Histologic examination was performed by single pathologists. Nasopharyngeal swabs (NPS) were obtained from the neonates within 24 h and at 48 h of birth.

RT-PCR: About 300 μL of whole sample was used for RNA extraction by silica membrane–based spin column technology using Qiagen viral RNA Minikit, (Qiagen catalogue no. 74104, USA). The RNA samples were stored at $-20\,°C$ for further analysis. Extracted RNA were run in real-time PCR machine (Biorad CFX 96) using one-step real-time RT-PCR kits (targeting Orf1ab and N gene, primer-probe mix) approved by ICMR, Govt of India. Positive control for amplification control and no template control to check out contamination were used for each run.

2.1. Statistical analysis

Continuous variables were expressed as mean $\pm$ SD. Categorical variables were expressed as number and frequency. Independent *t*-test was used to compare the continuous variables of normally distributed samples of the two group and p-value <0.05 was considered statistically significant. All statistical analysis was done with MedCalc Statistical software version 19.2.6 (MedCalc Sofware bvba, Ostend, Belgium; http://www.medcalc.org; 2020).

3. Result

A total of 50 pregnant women with 50 newborns (1 twin) were recruited in the study. Table 1 summarizes the characteristics of COVID-19 patients in labor or indication for labor. Mean age of the studied

Table 1
Clinical characteristics of the covid-19 patients for indication of labor.

Clinical features	Number	Mean ± SD/Frequency
Age (years)		28.19 ± 5.07
Gestational age at delivery (weeks)		
<34	2	4%
34–37	10	20%
>37	38	76%
Disease severity		
Mild	38	76%
Moderate	6	12%
Severe	6	12%
Disease to delivery interval (days)	8.2 ± 11.5	
Mode of delivery		
Caesarean	43	86%
Vaginal	7	14%
Fetal weight (kg)		
<1.5	4	8%
1.5–2	1	2%
2–3	27	54%
>3	18	36%
Pregnancy complication		
GDM	5	10%
Hypothyroidism	11	22%
Heart disease	2	4%
PIH	5	10%
Liver disease	9	18%
Coagulation disorder	2	4%
Neonatal complication		
MAS	1	2%
DIC	1	2%
HIE	1	2%
MODS	2	4%
RDS	3	6%
Placental pathology		
MVM	0	
FVM	25	50%
AIP	2	4%
CIP	0	
Others	37	74%
Cord blood		
IgG	12	24%
IgM	3	6%
Neonatal NP swab at birth	0	
Neonatal NP swab after 24 h	0	
High vaginal swab	0	
Placental swab	0	
Fetal membrane swab	1	
Amniotic fluid	1	
Breast milk	0	

GDM-gestational diabetes mellitus; PIH- pregnancy induced hypertension; MAS-meconium aspiration syndrome; HIE-hypoxic ischemic encephalopathy; DIC-disseminated intravascular coagulopathy; MODS-multiorgan dysfunction syndrome; RDS-respiratory distress syndrome; MVM-maternal vascular malperfusion; FVM-fetal vascular malperfusion; AIP-acute inflammatory pathology; CIP-chronic inflammatory pathology; IgG-immunoglobulin G; IgM-immunoglobulin M; NP- nasopharyngeal.

population was 28.19 ± 5.07 years. Among the 50 COVID-19 positive women, 38 had mild disease, 6 had moderate disease and six had severe disease. Most of the women presented at or near term except the two, one presented at 28 + 5 weeks and another at 31 weeks of gestation. Mean disease to delivery interval was 6.9 ± 12.8 days. Obstetric complications were observed in 34 women. Cesarean delivery was more frequent compared to vaginal delivery (43 vs 7). Very low birth weights were observed in only 4 newborns. Neonatal complications were seen in 8 newborns. Two had developed multiorgan dysfunction (one due to prematurity and hypoxic ischemic encephalopathy and one because of early onset neonatal sepsis), whereas three had respiratory distress syndrome (two of them were of low birthweight and one had meconium aspiration). None of them had developed COVID related pneumonia. Thirty nine babies were separated from their mothers and were either expressed breast milk fed or formula fed for 1st week of life. Eleven babies were roomed in immediately after birth on maternal request and were breastfed using appropriate preventive measures.

Placental, fetal membrane, amniotic fluid, vaginal secretion and breast milk were tested for SARS-CoV-2 infection by RT-PCR. All samples were negative for COVID-19 infection. Only one fetal membrane and amniotic fluid sample were positive for SARS-CoV-2 of a woman with mild COVID disease who delivered vaginally at 38 weeks gestation, though her other samples including neonatal NPS RT-PCR and cord blood IgG and IgM were negative. None of the neonates tested positive on nasopharyngeal RT-PCR for SARS-CoV-2 within 24 h and at 48 h of birth. Cord blood was analyzed for the presence of IgM and IgG antibody against COVID-19. IgG antibody was detected in 12 samples. COVID IgM was detected in three neonates. One of the three was born to a mother with severe disease and other two were born to the mothers with mild disease. Details of the cases with any of the sample positive for RT-PCR for SARS-CoV-2 are summed up in Table 2.

The most common placental histopathological lesion was fetal vascular malperfusion (FVM) in 50% followed by features of acute inflammatory pathology in 2%.

Table 3 compares the clinical characteristics of the cases with and without cord blood IgG positive status. Maternal severe COVID-19 disease and increasing disease to delivery interval were significantly associated with positive cord blood IgG (27.2% vs 6%, P < 0.01). There was no significant differences in maternal biochemical markers among the two groups except the ferritin level. Placental histology was also found similar in both the groups.

4. Discussion

Result of this study suggests that vertical transmission of COVID-19 is possible. Among the expecting mothers with confirmed COVID-19 infection, only one mother had amniotic fluid and fetal membrane sample positive. 12 newborns were screened positive for cord blood IgG and three for IgM. However none of the newborn had a positive RT-PCR result within 24 h and at 48 h of birth.

Vertical transmission could be possible with one of these mechanism: through placenta/umbilical cord; exposure to cervicovaginal secretions during delivery or via breast milk. Li et al. [5] demonstrated that the ACE2 receptor used by SARS-CoV-2 was widely distributed in the placental tissue and fetal organs. From the placenta, the virus can enter to fetal circulation through chorionic villi. However, data on placental transfer of COVID -19 is contradictory. Few studies refuting the vertical transmission and placenta affection by SARS-CoV-2 [7–10], whereas others have confirmed the transmission through placenta [11–13]. The most common placental lesion reported to be associated with COVID-19 infection is fetal vascular malperfusion (mostly intramural fibrin deposition) [13]. Consistent with previous studies, in present study too most common placental lesion was FVM (50%). This fetal vascular malperfusion might be related to hypercoagulability associated with COVID-19, however, as the lesions were low grade and the infants tested negative, these findings may be unrelated. In present study, one amniotic fluid and

Table 2
Clinical characteristic of mothers having neonates with any of the sample positive for RT-PCR SARS-CoV-2 or cord blood IgM positive status.

Clinical features	Case 1	Case 2	Case 3	Case 4
Gestational age (in weeks)	30 + 6	39 + 5	37 + 5	38
Parity	G1	G2	G2	G4
Disease severity	Mild	Mild	Severe	MILD
Antenatal high risk factor	Twin,PTL	Hypothyroid	Cardiac (MVR)	IHCP
Disease to delivery interval (in days)	6	2	10	7
Mode of delivery	LSCS	NVD	LSCS	NVD
Fetal weight (in Kg)	1.47, 0.724	3.59	2.7	2.85
Postpartum complications		None	None	none
Neonatal complication	One baby died 1 day after birth	Nil	Nil	Nil
Chest X-ray	NA	NA	Consolidation	NA
CRP (mg/L)	5.71	34.2	10.2	2.8
IL-6 (pg/mL)		9.6	328.7	1.6
Ferritin (mcg/L)	55.16	52.06	507.2	108.1
Procalcitonin (ng/mL)	0.059	0.042	0.14	0.075
D-dimer (ng/mL)	1.92	5.85	2.21	1.8
NLR	4.34	3.38	8.06	2.8
LDH (U/L)	464.1	667.63	981.2	831
RT-PCR				
Placenta	Neg	Neg	Neg	Neg
Fetal membrane	Neg	Neg	Neg	Positive
Amniotic fliud	Neg	Neg	Neg	Positive
Vaginal fluid	Neg	Neg	Neg	Neg
Neonatal NP/OP				
At 24 h	Neg	Neg	Neg	Neg
At 48 h	Neg	Neg	Neg	Neg
Breast milk	Neg	Neg	Neg	Neg
Placental HPE	FVM, perivillous fibrin,	FVM, perivillous fibrin	FVM, increased syncytial knots	Nothing specific
Cord blood				
IgG	Positive	Negative	Positive	Negative
IgM	Positive	Positive	Positive	Negative

G-gravida; PTL-preterm labour; MVR-mitral valve regurgitation; IHCP- intrahepatic cholestasis of jaundice; LSCS- lower segment cesarean section; NVD-normal vaginal delivery; CRP- C-reactive protein; IL-6- interleukin-6; LDH-lactate dehydrogenase; NLR-neutrophil to lymphocyte ratio; RT-PCR- Reverse transcriptase polymerase chain reaction; NP/OP- nasopharyngeal/oropharyngeal; HPE-histopathological examination; FVM-fetal vascular malperfusion; IgG-immunoglobulin G; IgM-immunoglobulin M.

fetal membrane samples were tested positive for SARS-CoV-2 RT-PCR, but other samples including cord blood IgG/IgM and neonatal RT-PCR were negative. However placenta did show low grade lesion suggestive of FVM or inflammatory pathology. This suggests that placental affection do not always lead to fetal infection.

Detection of antibodies against COVID-19 in cord blood also supports the transplacental transmission of the infection. Dang et al. had reported elevated IgM in the neonate's blood sample drawn 2 h after birth which suggests that the neonate was infected in utero as IgM antibodies usually do not appear until 3–7 days after infection and IgM antibodies cannot be transferred to the fetus via the placenta [2]. Transplacental transfer of IgG can occur and appears later than IgM, hence elevated IgG level in newborn reflects passive immunity from mother or neonatal infection. In the present study, IgG was detected in cord blood of 12 neonates (24%) and IgM in 3 neonates (6%). IgM is not transferred

Table 3

Comparing maternal biochemical markers and histology of placenta among neonates with IgG positive and negative status.

Maternal biochemical markers	Neonates with IgG Positive	Neonates without IgG	p-value
Disease severity (severe)	3 (27.2%)	3 (7.6%)	0.01
Disease to delivery interval (in days)	10.18 ± 13	7.2 ± 12.5	0.03
NICU admission	2 (18.18%)	5(12.8%)	
Ferritin (mcg/L)	281.7 ± 228.2	127.7 ± 141.2	0.01
Procalcitonin(ng/mL)	0.49 ± 0.63	1.3 ± 3.7	0.45
LDH (U/L)	639.9 ± 270.2	651.3 ± 256.1	0.89
D-dimer (ng/mL)	2.9 ± 1.8	3.8 ± 5.08	0.55
IL-6 (pg/mL)	48.5 ± 96.5	75.9 ± 192	0.65
CRP (mg/L)	26.3 ± 21.2	34.3 ± 61.2	0.67
NLR	4.6 ± 2.3	4.1 ± 2.9	0.31
AIP	0/11	2/39	
CIP	0	0	
MVM	0	0	
FVM	7/11	18/39	
OTHERS	8/11	29/39	

NICU- neonatal intensive care unit; CRP- C-reactive protein; IL-6- interleukin-6; LDH- lactate dehydrogenase; NLR-neutrophil to lymphocyte ratio; MVM-maternal vascular malperfusion; FVM-fetal vascular malperfusion; AIP-acute inflammatory pathology; CIP-chronic inflammatory pathology; IgG-immunoglobulin G; IgM-immunoglobulin M.

transplacentally through normal and healthy placenta, however, it can be transferred from mother to fetus in presence of viral cytotoxic effects or severe inflammation of the birth canal [14]. Hence, presence of IgM antibody cannot be assumed as an evidence of in-utero transfer of SARS-CoV-2 infection. In this study, IgM was detected in the cord blood of three neonates, but other samples including fetal membrane, placental, amniotic fluid and cervico-vaginal secretions for COVID-19 were negative. Secondly placental histology in all the three cases demonstrated FVM and perivillous fibrin deposition which were suggestive of low-grade lesion. So findings of this study do not support the irregular transfer of IgM through birth canal.

Recently Flannery et al. had detected IgG in cord blood of 87% of neonates born to mothers with COVID-19 disease, though IgM was not detected in any of the babies [15]. He also demonstrated positive correlation between maternal and cord antibody concentration and increasing duration between onset of maternal infection and time of delivery. In present study too, we found positive correlation of cord blood antibody with increasing disease to delivery interval and disease severity. We did not observe significant association with maternal biochemical markers, mode of delivery or preterm delivery.

Evidence of presence of SARS-CoV-2 in cervicovaginal seretions is controversial, few studies reported its detection in cervicovaginal fluid [16,17] whereas others had not identified SARS-CoV-2 in cervicovaginal secretions [18,19]. Adding to the controversy, we too did not identify SARS-CoV-2 in cervicovaginal secretions. So we claim that vertical transmission through exposure to cervicovaginal secretion during delivery is very unlikely. This findings also indicate that vaginal delivery does not increase the risk of vertical transmission. Consistent with the previous literature, SARS-CoV-2 was not detected in the breast milk of any of the lactating women, hence breast milk may not be the source of infection to the infant [20]. This findings also reaffirms the continuation of breastfeeding by women with COVID-19 given its known benefits.

Recently a systemic review analyzing the risk of vertical transmission of SARS-CoV-2 which included 1787 pregnancies, had demonstrated 2.8% positivity rate among infants born to COVID-19 positive mothers [21]. However authors warranted further studies on the mechanism underlying the variability of vertical transmission from one pregnancy to another. Sinaci et al. had found one placental and two of the vaginal secretion samples positive for SARS-CoV-2 in his study [22]. He also detected IgG-IgM antibody in two newborns within 24 h of delivery. However, none of these neonates were screened positive for COVID -19

through NPS RT-PCR. As they tested IgG-IgM together, source of antibodies (maternal or neonatal) could not be clarified. They concluded that detection of the virus in the placenta or vaginal secretions may not be associated with neonatal infection.

As per the classification system suggested by Sah et al., a confirmed case of congenital infection is defined by identifying the virus via RT-PCR of cord blood within 12 h of birth in neonate born to covid-19 positive mother, or in amniotic fluid obtained prior to rupture of membrane and probable case by detection of the virus by PCR in NPS at birth (collected after cleaning baby) and placental swab from fetal side of placenta. He suggested that detection of IgM against SARS-CoV-2 in cord blood denotes possible intrauterine infection [23]. In present study, one amniotic fluid and fetal membrane sample was positive for SARS-CoV-2, but other samples including cord blood IgG/IgM and neonatal RT-PCR were negative, and IgM was detected in cord blood of three neonates. Furthermore, none of the newborns screened positive via NPS RT-PCR within 48 h of birth or developed COVID-19 disease during two weeks follow-up. This indicates that though the possibility of vertical transmission of SARS-CoV-2 cannot be ruled out, none of the babies developed COVID-19 disease.

4.1. Limitations of the study

This study has few limitations. First, sample size is small and from single center so results may not be generalizable. We did not perform anal swab RT-PCR in neonates which would have helped in confirming the congenital infection as shedding of virus in feaces have been demonstrated in the literature.

4.2. Strength of the study

This is a prospective study and all possible mechanism of vertical transmission have been curated. We performed comprehensive testing of all biological samples for vertical transmission including the cord blood immunoglobulin and followed up the neonates. We examined the placenta for any COVID-19 related change in histology and correlated with neonatal outcome. To conclude, presence of SARS-CoV-2 in amniotic fluid and fetal membrane in a pregnant woman and IgM in the cord blood suggest that virus can be transmitted from mother to fetus either through placenta or umbilical cord. However, presence of SARS-CoV-2 in fetal or placental tissue did not result in neonatal COVID-19 disease. Absence of SARS-CoV-2 in cervicovaginal secretion or breast milk makes this route of vertical transmission unlikely. Passive immunity against covid-19 is conferred to neonates is evident from detection of IgG in cord blood. This finding has greater clinical implication as large number of expecting mothers are being vaccinated.

Author's contribution

Data collection and analysis was done by SJ and AS. Concept, designing and writing of manuscript were done by SJ. MA was involved in initial drafting of manuscript. PB reviewed all the slides and gross findings of placenta. BKC was involved in neonatal care. BKP was involved in processing and furnishing of RT-PCR reports. MM contributed in running cord blood samples and providing reports. All the author have read and approved the final version of manuscript.

Synopsis

Vertical transmission of covid-19 is possible though the risk is low.

Ethical approval and consent to participate

Study had been approved by institute ethical committee (AIIMS/Pat/IEC/2020/573). Verbal consent had been obtained from the study participants. Ethical committee approved this procedure.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Declaration of competing interest

Authors declare that they have no competing interest.

Abbreviations

WHO   World health organization
MERS   Middle East respiratory syndrome
SARS CoV-2 Severe acute respiratory syndrome corona virus-2
COVID-19   Coronavirus disease 2019
VTM   Viral transport media
NPS   Nasopharyngeal swab
RT-PCR   Reverse transcriptase polymerase chain reaction
NICU   Neonatal intensive care unit
ELISA –   Enzyme linked immunoassay
IgG   Immunoglobulin G
IgM   Immunoglobulin M
FVM   fetal vascular malperfusion

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