INTRAUTERINE TRANSMISSION OF SARS-COV-2 INFECTION IN A PRETERM INFANT

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Abstract: We present a preterm infant who developed a fever and mild respiratory disease on the second day of life. Infant severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nasopharyngeal testing was positive at 24 and 48 hours of life. Placenta histopathology revealed SARS-CoV-2 infection by electron microscopy and immunohistochemistry. Further understanding of the risk factors that lead to *in utero* transmission of SARS-CoV-2 infection is needed.

Key Words: neonatal, vertical transmission, coronavirus

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the viral agent that causes coronavirus 2019 disease (COVID-19), first appeared in Wuhan, China, in December 2019. The first confirmed case of COVID-19 was reported on January 20, 2020, in the United States, and the World Health Organization declared the disease a public health emergency of international concern on January 30, 2020. This single-stranded RNA virus is highly contagious and primarily transmits via droplet, contact and aerosol transmission. There are limited data on mother-to-infant transmission of SARS-CoV-2. However, studies including large case series of pregnant women with COVID-19 from Wuhan, China, suggested that transmission of SARS-CoV-2 *in utero* or intrapartum is unlikely, as amniotic fluid, cord blood and breast milk samples from the mothers have all been negative for SARS-CoV-2.[1] Despite these studies, a growing number of suspected congenital or intrapartum-acquired neonatal SARS-CoV-2 cases have been reported.[2–6]

CASE SUMMARY

A 34 weeks' gestation, large for gestational age female infant was born to a 37-year-old gravida 4, para 3 woman via vaginal delivery. Pregnancy was complicated by maternal Class B diabetes mellitus, and obesity (body mass index: 55). The mother's HbA_{1C} was 5.9%, and she was treated with insulin during pregnancy. She also received three doses of benzathine penicillin during the second trimester of pregnancy for late latent syphilis. She was admitted to the hospital for evaluation of preterm labor since she had a 1-day history of intermittent sharp back pain, fever and diarrhea. Her nasopharyngeal swab specimen tested positive for SARS-CoV-2 infection by reverse-transcriptase polymerase chain reaction (RT-PCR) performed on the XPERT XPRESS SARS-CoV-2 RT-PCR (Cepheid, Sunnyvale, CA). She was placed on contact, droplet and airborne isolation and instructed to wear a rectangular mask during the entire hospital stay. Labor was augmented with oxytocin on the third day after hospitalization after she developed premature preterm rupture of membranes 8 hours before delivery. The amniotic fluid was clear. Ampicillin was given 6 hours before the delivery for group B *Streptococcus* prophylaxis.

The delivery was attended by the Neonatal Intensive Care Unit (NICU) Team. Apgar scores were 7 and 9 at 1 and 5 minutes of life, respectively. Cord arterial blood gas was mildly acidotic (pH 7.18, base deficit −9.6). As part of the labor and delivery protocol for mothers with COVID-19, delayed cord clamping, and skin-to-skin contact were not performed. The infant was immediately separated from the mother and placed on a radiant warmer >6 feet away, and the infant was then transferred to the NICU within 30 minutes for management of prematurity, glucose monitoring and SARS-CoV-2 exposure.

In the NICU, the infant was bathed before routine neonatal care. The birth weight was 3280 g. The infant's vital signs and physical examination were normal, and there was no respiratory distress. Serial blood glucose measurements were normal, and the initial hematocrit was 53%. A rapid plasma reagin test was negative, confirming adequate treatment of the mother for latent syphilis in pregnancy. The infant developed significant indirect hyperbilirubinemia within the first 24 hours of life. Serum transaminases were not elevated. Serum bilirubin concentrations increased significantly despite aggressive phototherapy, with evidence of hemolytic anemia and reticulocytosis (Table, Supplemental Digital Content 1, http://links.lww.com/INF/E56). A dose of intravenous immunoglobulin was given to prevent exchange transfusion for suspected ABO incompatibility.

The infant's nasopharyngeal swab was positive by RT-PCR for SARS-CoV-2 at 24 and 48 hours of life. The infant developed fever and respiratory distress [mild subcostal retractions, tachypnea and hypoxia (lowest oxygen saturation on room air of 78%)] on the second day of life and required nasal cannula at 1 L/min flow with minimal oxygen supplementation. Because of the respiratory distress and neonatal fever, blood and cerebrospinal fluid bacterial cultures, and surface, blood and cerebrospinal fluid herpes simplex virus DNA PCR were obtained. Intravenous ampicillin, gentamicin and acyclovir were started. Chest radiograph was unremarkable. Serial complete blood counts showed elevated neutrophils and immature: total neutrophil ratio, but lower lymphocyte count (Table, Supplemental Digital Content 1, http://links.lww.com/INF/E56). Bacterial cultures and herpes simplex virus PCR were negative after 48 hours, and antibiotics and acyclovir were discontinued. Respiratory signs resolved 3 days after their onset and the infant was weaned to room air by day 5 of life. The infant received a small amount of expressed colostrum and breast milk during the first 3 days of life. Nasopharyngeal RT-PCR for SARS-CoV-2 was still positive on day 14 of life. The infant was discharged home with her mother in good clinical condition on day 21 of life.

Histopathologic examination of the placenta, which was large for gestational age, showed patchy histiocytic (CD68 positive) intervillositis and villitis associated with villous karyorrhexis and necrosis (Fig. 1A), focal basal chronic villitis, focal parabasal infarct and features of meconium exposure in the fetal membranes. Immunohistochemistry for SARS-CoV-2 using mouse monoclonal anti-SARS-CoV nucleocapsid protein antibody[7] (Catalog no. 40143-MM05, Sino Biological, Chesterbrook, PA), performed on an Omnis immunostainer (Agilent Dako, Santa Clara, CA), showed cytoplasmic staining in the syncytiotrophoblastic cells (Fig. 1B). Ultrastructural examination by transmission electron microscopy showed 89 to 129 nm diameter structures consistent with viral

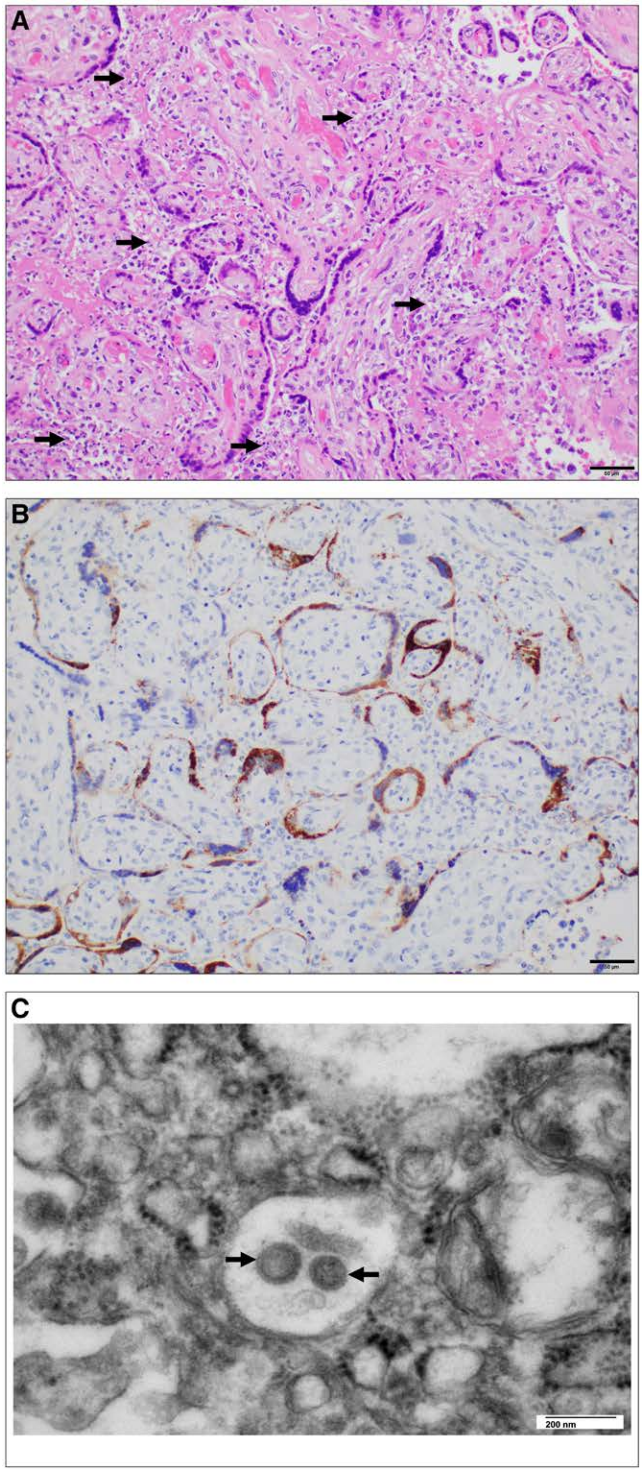

FIGURE 1. Histopathologic features of the placenta included histiocytic intervillositis (arrows, **A**, hematoxylin and eosin, ×200 magnification), immunohistochemical staining for SARS-CoV nucleocapsid protein in the cytoplasm of the syncytiotrophoblastic cells (**B**, immunoperoxidase, ×200 magnification), and viral-like particles (arrows) in membrane-bound cisternal spaces in the syncytiotrophoblastic cells (**C**, transmission electron microscopy, ×40,000 magnification).

TABLE 1. Clinical Laboratory Results

	Reference	24 h	40 h	48 h	72 h
Hemoglobin (g/dL)	14.5–22.5		18.3	15.9	16.3
Hematocrit (%)	45–67		52.3	45.6	46.7
Red cell count (×106 per mm^3)	4.0–6.6		5.14	4.38	4.61
Mean corpuscular volume (fl)	90–101		101.8		101.3
Mean corpuscular hemoglobin (pg)	31–37		35.6		35.4
White cell count (×103 per mm^3)	5–21		1.146	9.93	13.19
Differential count (%)					
Neutrophils	24–61		75	61	52
Bands	8–16		1	11	13
Lymphocytes	30–53		13	6	23
Monocytes	4–18		6	14	18
Eosinophils	1–7		2	1	3
Basophils	0–1		0	0	0
Metamyelocytes			1	4	1
Myelocytes			2	3	1
Promyelocytes					
Immature/total ratio				0.23*	0.22*
Platelets (×103 per mm^3)	174–404		192	159	173
Reticulocyte count per mm^3	3200-14700		53000		47500
Reticulocyte (%)	1%–6%		10.9*		11.1*
Sodium (mmol/L)	135–145	136		133	140
Potassium (mmol/L)	3–5.5	4.5		4.7	4.8
Chloride (mmol/L)	98–109	105		104	109
Bicarbonate (mmol/L)	22–26	23		17†	21
Glucose random (mg/dL)	60–100	71		73	71
Blood urea nitrogen (mg/dL)	4–19	9		5	9
Creatinine (mg/dL)	0.31–0.88	0.9		0.78	0.58
Bilirubin total (mg/dL)	0.2–6	11.8*	17.1*	14.7*	11
Bilirubin direct (mg/dL)	0-0.3	0.3			
Alkaline phosphatase (U/L)	≤231		146		
Aspartate aminotransferase (U/L)	10–35		64		
Alanine aminotransferase (U/L)	10–35		10		

*The value in the patient was above the normal range.
†The value in the patient was below the normal range.

particles clustered within membrane-bound cisternal spaces in the syncytiotrophoblastic cells (Fig. 1C).[8] Amniotic fluid and breast-milk PCR, and cord blood antibody testing is not available at our hospital.

DISCUSSION

This report represents a case of congenital SARS-CoV-2 infection in an infant born via vaginal delivery to a mother with COVID-19 presenting primarily with gastrointestinal manifestations. It is unlikely that the respiratory distress observed in this infant was due to prematurity since it did not start until the second day of life. Although the infant had an elevated neutrophil count, the lymphocyte count was decreased as has been described in adults with COVID-19. Viral hepatitis was excluded by the absence of elevated liver transaminases.

Early reports of obstetric and neonatal outcomes of pregnant women with COVID-19 suggested that SARS-CoV-2 infection in pregnancy results in infants with no clinical features of infection and negative nasopharyngeal testing.[9] However, there are now an increasing number of neonatal cases with possible congenital or intrapartum-acquired neonatal SARS-CoV-2 infection. To date, 8 infants with SARS-CoV-2 virus infection proximate to delivery have been reported.[2–6] These reports include a case series of 3 infants (2 term infants and a preterm infant) with fever and pneumonia and positive nasopharyngeal and anal SARS-CoV-2 RT-PCR on day of life 2 and 4,[2] a preterm infant with fever and initially negative SARS-CoV-2 PCR at delivery but positive at 24 hours of life and during the second week of life,[3] a preterm infant who developed respiratory disease with positive SARS-CoV-2 RT-PCR at 16 and 48 hours of life,[4] and a preterm infant with probable congenital

infection with positive SARS-CoV-2 PCR from nasopharyngeal, blood, stool and placenta.[5] Recently, 2 asymptomatic neonates with positive SARS-CoV-2 RT-PCR after 24 hours, and after 7 days have been reported.[6] Of note, SARS-CoV-2 spike protein mRNA was detected on the fetal side of the placenta of women who delivered these neonates.

The symptomatic preterm infant described in this report demonstrated SARS-CoV-2 virus in both placental tissue and nasopharyngeal samples, with exclusion of bacterial and other viral neonatal infections. Although the histologic placental findings of histiocytic intervillositis and chronic villitis are not specific to SARS-CoV-2 infection, the presence of cytoplasmic staining for the SARS-CoV-2 nucleocapsid protein by immunohistochemistry and demonstration of viral particles by electron microscopy in the syncytiotrophoblastic cells strongly suggest in utero transmission.

A classification system for SARS-CoV-2 infection in pregnant women, fetuses and neonates has been described by Shah et al.[10] This classification includes placental PCR swabs and infant nasopharyngeal swabs at birth as probable evidence of congenital SARS-CoV-2. It does not consider ultrastructural demonstration of coronavirus particles or immunohistochemical detection of SARS-CoV-2 in the placenta. However, the infant described here represents congenital infection given the immunohistochemical and ultrastructural evidence of SARS-CoV-2 infection in the fetal cells of the placenta, criteria that we believe should be added to the classification system to confirm intrauterine transmission.

Overall, intrauterine transmission of SARS-CoV-2 appears to be a rare event. In the infant described, transmission could have occurred either due to ascending infection with premature rupture of membranes and primary involvement of the maternal gastrointestinal tract, or by hematogenous spread if the mother was viremic during her initial infectious period. Further studies are needed to determine the risks of vaginal delivery of mothers with SARS-CoV-2.

Additional studies on the mechanisms and risk factors of in utero SARS-CoV-2 transmission and the outcomes of congenital infection are urgently needed. In particular, the susceptibility to intrauterine transmission by gestational age and the relation to maternal active disease needs to be explored. Improving access to molecular testing of amniotic fluid and breast milk, cord blood antibody testing and establishing biorepositories for respiratory and nonrespiratory samples from exposed infants will enable investigators to further describe the epidemiology of congenital and neonatal disease in the setting of maternal SARS-CoV-2 infection.

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