Visualization of SARS-CoV-2 virus invading the human placenta using electron microscopy

Gabriela N. ALGARROBA, MD[1], Patricia REKAWEK, MD[1], Sevan A. VAHANIAN, MD[1], Poonam KHULLAR, MD[2], Thomas PALAIA, MS[3], Morgan R. PELTIER, PhD[3], Martin R. CHAVEZ, MD[1], Anthony M. VINTZILEOS, MD[1]

Author affiliations

1. Department of Obstetrics and Gynecology, NYU Langone Health, NYU Winthrop Hospital, NYU Long Island School of Medicine
2. Department of Pathology, NYU Langone Health, NYU Winthrop Hospital, NYU Long Island School of Medicine
3. Department of Foundations of Medicine, NYU Langone Health, NYU Winthrop Hospital, NYU Long Island School of Medicine

Correspondence

Martin R Chavez, MD
120 Mineola Boulevard, Suite 110
Mineola, NY, 11501
Email: martin.chavez@nyulangone.org
Phone: 516-663-9539
Fax: 516-742-7841

Word counts: 1285

Conflicts: The authors report no conflict of interest

Funding: None

INTRODUCTION

The outbreak of the novel severe acute respiratory syndrome coronavirus (SARS-CoV-2), which results in development of coronavirus disease (COVID-19) has been associated with significant morbidity and mortality. The risk of vertical transmission from infected pregnant women to their fetuses is controversial. Recent studies have revealed the possibility of vertical transmission (1, 2), contrary to previous reports of no evidence of vertical transmission of SARS-CoV-2 (3). Whether vertical transmission occurs and if so, with which frequency remains unknown (4).

We present a case of rapid clinical deterioration in a woman at 28 weeks' gestation due to severe COVID-19 infection. Using electron microscopy to evaluate for potential viral transmission in the placenta, we visualized and identified coronavirus virions invading into syncytiotrophoblasts in placental villi. To our knowledge, this is the first report demonstrating direct evidence of SARS-CoV-2 virus invasion in placental tissue and placental infection associated with SARS-CoV-2 virus.

Clinical Presentation

A 40-year-old Hispanic female, G3P2002, at 28 weeks and 4 days, with no significant past medical history, presented to the emergency department with worsening shortness of breath, cough, and hypoxia in the setting of a known COVID-19 infection, on day 2 of 5 of an azithromycin course. She was promptly admitted with the diagnosis of sepsis pneumonia secondary to COVID-19 infection.

Ten hours after the initial presentation, her clinical condition deteriorated with progressively increasing oxygen requirements. She was intubated, sedated, and started on a norepinephrine infusion due to hypotension in order to maintain appropriate perfusion for the placenta. Antenatal corticosteroids for fetal lung maturity were administered in anticipation of a preterm delivery. Therapeutic anticoagulation with heparin was initiated due to risk of venous thromboembolism in the setting of severe COVID-19 infection with elevated D-dimer. She received a one-time dose of 400 mg

tocilizumab, an interleukin 6 receptor antagonist, while awaiting regulatory permission to start use of the antiviral remdesivir. On HD 4, she developed a metabolic acidosis (pH 7.19, pCO2 26 mmHg, pO2 338 mmHg, HCO3 9.9 mmol/L, base deficit 17 mmol/L) and, despite a bicarbonate infusion, she continued to deteriorate. The decision was made to proceed with delivery to optimize maternal treatment and decrease fetal morbidity. She received a magnesium sulfate 4 g bolus for fetal neuroprotection. An uncomplicated repeat cesarean delivery was performed in a negative pressure operating room with all personnel in personal protective equipment of a female infant weighing 2 lbs and 15 oz (1340 grams). The cord blood arterial gas was pH 7.26, PCO2 46, PO2 38, HCO3 20.6 and base deficit 7. APGARS were 3, 5, and 6 at 1, 5, and 10 minutes, respectively. PCR testing was not performed on the placenta or amniotic fluid.

Postoperatively, the patient received a ten day course of remdesivir. She recovered well with progressively lower oxygen requirements and resolution of metabolic acidosis. The patient was discharged home on POD 10 with therapeutic enoxaparin for 12 weeks. The infant's COVID-19 testing was negative on day of life (DOL) 2 and 3.

Laboratory Methods and Analysis

Patients with suspected COVID-19, including infants, are tested via SARS-CoV-2 PCR of a nasopharyngeal swab, using the Cepheid Xpert™ Xpress SARS-CoV-2 RT-PCR assay under EUA as per our institution's policy. All placentas from COVID-19 positive mothers are submitted for gross and histologic evaluation in our institution. In this case, the placenta was submitted to the pathology laboratory without fixative; fresh tissue was taken, using appropriate personal protective gear, under the Fisher Scientific Safety Flow Lab Fume Hood. Two 1 mm fragments were taken, one from chorionic villi deep within the placental parenchyma and one from the decidua on the maternal surface. The tissue was fixed in 4% glutaraldehyde for electron microscopic evaluation. The placenta was then fixed in 10%

buffered formalin for 72 hours prior to sectioning. Ten representative, 3 mm thick tissue sections were submitted from the placental parenchyma, membranes and umbilical cord for histologic evaluation.

Given the severity of the patient's clinical course, suspected viremia, and the presence of ACE2 receptors in the placenta (5), transmission electron microscopy (TEM) was utilized as an opportunity to learn more about potential viral transmission in the placenta. To perform the TEM, placental tissue samples were fixed in 4% glutaraldehyde buffered in 0.1 M sodium cacodylate buffer, pH 7.5, washed in sodium cacodylate buffer, post-fixed in buffered 1% osmium tetroxide, en-bloc stained with a saturated solution of uranyl acetate in 40% ethanol, dehydrated in a graded series of ethanol, infiltrated in propylene oxide with Epon epoxy resin (LADD LX112, Ladd industries, Burlington, VT), and embedded. The blocks were sectioned with a Reichert Ultracut^{TM} microtome at 70 nm. The resulting grids were then post-stained with a 1% aqueous uranyl acetate followed by 0.5% aqueous lead citrate and scoped on a Zeiss EM 900 transmission electron microscope retro-fitted with an SIA L3C digital camera (SIA, Duluth, GA).

FINDINGS

The placenta weighed 271 g (75th to 90th percentile). Sections showed mature chorionic villi with focal villous edema and an area of decidual vasculopathy on the maternal surface. Survey from a placental thick section showed the terminal villi containing fetal blood vessels (Figure 1). This area was used for the transmission electron microscopy and contained syncytiotrophoblasts, fibroblasts, endothelial cells, and fetal red blood cells. A single virion was visible invading a syncytiotrophoblast (Figure 2). This virion was again visualized under a higher magnification (Figure 3). A single virion was also visualized in a microvillus (Figure 4). Additionally, at the highest magnification of the mesenchymal core of the terminal villus, likely in the cell processes of fibroblasts, a single virion was visible in one field (Figure 5) as well as two virions in another (Figure 6).

DISCUSSION

This is the first visualization of the SARS-CoV-2 virus in the human placenta. Using electron microscopy, we were able to identify virions invading syncytiotrophoblasts in placental villi. In addition, we identified SARS-CoV-2 virions in placental villi in the cell processes of fibroblasts. It appears that the cells are fibroblasts which may take the form of myofibroblasts as a result of response to injury or inflammation, in this case by the SARS-CoV-2 virus (6). Our findings further contribute to the evidence of placental infection with SARS-CoV-2; however, there was no evidence of fetal infection.

The risk of intrauterine transmission is of particular interest as the SARS-CoV-2 virus utilizes the ACE2 receptor for cell entry and it is known that there is expression of the ACE2 receptor in the human placenta (5). Two recently published studies have provided evidence for the potential for vertical transmission. In a report by Zamaniyan et al (1) there was evidence of potential intrauterine infection in a woman with severe COVID-19 disease who delivered at 32 weeks gestation as shown by positive RT-PCR tests for COVID-19 in amniotic fluid as well as repeat neonatal nasal and throat swabs; initial neonatal swabs, as well as vaginal secretions and umbilical cord blood were negative for COVID-19. In a study by Dong et al (2), a neonate born to a mother with COVID-19 infection of at least 20 days duration was shown to have positive IgM and IgG antibodies as well as elevated IL-6 and IL-10 cytokines at two hours of birth while the maternal vaginal secretions were negative for COVID-19. Although the infant was asymptomatic and had multiple negative nasopharyngeal swabs tested for SARS-CoV-2, in utero infection was suspected as IgM antibodies do not cross the placenta and the neonate mounted an innate immune response. There was additional evidence of vertical transmission supported by laboratory results which showed inflammation and liver injury. In contrast, other studies have reported no evidence of vertical transmission of COVID-19 (3). Future studies are warranted examining placental pathology and obstetric and neonatal outcomes to assess for risk of vertical transmission of SARS-CoV-2.

REFERENCES

1. Zamaniyan M, Ebadi A, Aghajanpoor Mir S, Rahmani Z, Haghshenas M, Azizi S. Preterm delivery in pregnant woman with critical COVID-19 pneumonia and vertical transmission. Prenatal Diagnosis (2020). Doi: https://doi-org.ezproxy.med.nyu.edu/10.1002/pd.5713
2. Dong L, Tian J, He S, Zhu C, Wang J, Liu C, et al. Possible Vertical Transmission of SARS-CoV-2 From an Infected Mother to Her Newborn. Jama. 2020.
3. Chen H, Guo J, Wang C, Luo F, Yu X, Zhang W, et al. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. Lancet (London, England). 2020;395(10226):809-15.
4. Lamouroux A, Attie-Bitach T, Martinovic J, Leruez-Ville M, Ville Y, Evidence for and against vertical transmission for SARS-CoV-2 (COVID-19), American Journal of Obstetrics and Gynecology (2020), doi: https://doi.org/10.1016/j.ajog.2020.04.039.
5. Valdes G, Neves LA, Anton L, Corthorn J, Chacon C, Germain AM, et al. Distribution of angiotensin-(1-7) and ACE2 in human placentas of normal and pathological pregnancies. Placenta. 2006;27(2-3):200-7.
6. Baum J, Duffy HS. Fibroblasts and myofibroblasts: what are we talking about? J Cardiovasc Pharmacol. 2011;57(4):376-379.

FIGURE LEGENDS

Figure 1: Placental thick section at 1 micron stained with toluidine blue showing the terminal villi containing fetal blood vessels (10X). This area was used for the transmission electron microscopy.

Figure 2: Transmission electron microscopy of a single virion visible invading a syncytiotrophoblast (30,000X).

Figure 3: Transmission electron microscopy of a single virion visible invading a syncytiotrophoblast at a higher magnification (50,000X).

Figure 4: Transmission electron microscopy of a single virion visualized in a syncytiotrophoblast microvillus (50,000X).

Figure 5: Transmission electron microscopy of the trophoblastic layer in the mesenchymal core of the terminal villus where a single virion was visible, likely in the cell processes of fibroblasts (50,000X).

Figure 6: Transmission electron microscopy of the trophoblastic layer in the mesenchymal core of the terminal villus where two virions were visible, likely in the cell processes of fibroblasts (50,000X).