Intrauterine Fetal Demise in the Third Trimester of Pregnancy Associated With Mild Infection With the SARS-CoV-2 Delta Variant Without Protection From Vaccination

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Background. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has a higher infection rate in pregnant women than age-matched adults. With increased infectivity and transmissibility, the Delta variant is predominant worldwide.

Methods. In this study, we describe intrauterine fetal demise in unvaccinated women with mild symptoms of SARS-CoV-2 Delta variant infection.

Results. Histology and elevated proinflammatory responses of the placenta suggest that fetal demise was associated with placental malperfusion due to Delta variant infection.

Conclusions. This study suggests that the Delta variant can cause severe morbidity and mortality to fetuses. Vaccination should continue to be advocated and will likely continue to reduce SARS-CoV-2 infection risks for pregnant women and their fetuses.

Keywords. COVID-19; Delta variant; intrauterine fetal demise; pregnancy; SARS-CoV-2.

The coronavirus disease 2019 (COVID-19) pandemic has been caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus and has led to >266 million cases worldwide, ~5.26 million of which have been fatal as of December 8, 2021. Pregnant women are particularly vulnerable to COVID-19 and have a 70% higher infection rate than age-matched adults [1]. Between January 22, 2020 and December 6, 2021, a total of 150,036 pregnant women were confirmed with COVID-19 infections in the United States alone. A polymerase chain reaction (PCR) positivity rate of 15.4% with SARS-CoV-2 was reported in women presenting in labor in New York City, regardless of symptoms [2]. Pregnant women with COVID-19 typically experience mild illness, and their births are often uncomplicated [3]. However, there have been reports of pregnancy complications such as pre-eclampsia, preterm birth, intrauterine fetal demise (IUFD), and stillbirth, as well as serious maternal illness that required intensive care, and even maternal death [4]. The recent Morbidity and Mortality Weekly Report [5] on November 19, 2021 indicated that a COVID-19 diagnosis during the delivery hospitalization was associated with an increased risk for stillbirth in the United States, with a stronger association when the Delta variant was predominant.

Severe acute respiratory syndrome coronavirus 2 has evolved rapidly, and novel genetic variants continue to emerge and challenge disease prevention and control. The Delta variant has become the predominant variant worldwide, with a prevalence of 98.8% in the United States as of August 24, 2021. The Delta variant has been reported to be more contagious, and some individuals still have breakthrough infections despite vaccination [6, 7]. A large-scale cohort study showed patients with the Delta variant had more risk of hospital admission compared with patients with the Alpha variant, indicating that the Delta variant in unvaccinated populations may lead to higher healthcare burdens [8]. In this study, we describe IUFD associated with placental malperfusion due to cytokine storm during the third trimester of a pregnancy complicated by mild Delta infection.

METHODS

Ethics Approval and Biosafety
This study has been approved by the University of Missouri institutional review board ([IRB] nos. 2063342 and 2025449) and proper consent was obtained. All work with live SARS-CoV-2 was performed in a biosafety level 3 laboratory in compliance with state and federal regulations and with the approval of the University of Missouri Institutional Biosafety Committee.

Placental Immunohistochemistry Antigen Staining
Sections of placenta were deparaffinized, rehydrated, and underwent antigen retrieval and endogenous peroxidase activity quenching, then blocked with 10% normal goat serum before incubation with SARS-CoV-2 spike-specific mouse monoclonal antibody (Invitrogen, Carlsbad, CA). Sections were treated with a biotinylated goat antimouse immunoglobulin (Ig)G polyclonal secondary antibody, and ABC reagent (Vector Laboratories, Burlingame, CA) was applied following the manufacturer’s instructions. Sections were counterstained with hematoxylin, washed, dehydrated, and covered with coverslip.

Received 18 October 2021; editorial decision 20 December 2021; accepted 11 January 2022; published online 13 January 2022.
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The Journal of Infectious Diseases © 2022;XX:1–6
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https://doi.org/10.1093/infdis/jiac007
Viruses
The prototype SARS-CoV-2 virus isolate, USA-WA1/2020 (WA1; NR-52281), was obtained through BEI Resources. MU8944NPS (Delta-142G) and MU8946NPS (Delta-142D) were isolated from 2 COVID-19-positive patients’ nasopharyngeal swabs in Missouri.

Ribonucleic Acid Extraction
Supernatant from homogenized placental tissue were used for ribonucleic acid (RNA) extraction. Viral RNA was extracted using the RNaseasy Kits with RNase-Free DNase set (QIAGEN, Germantown, MD) following the manufacturer’s protocol.

Viral Ribonucleic Acid Quantification and Sequencing
The quantitative real-time PCR (qRT-PCR) was carried out using SARS-CoV-2 primer/probe sets (Integrated DNA Technology, Coralville, IA). The protocol was adapted from the Centers for Disease Control and Prevention’s RT-PCR Panel for detection of 2019-novel coronavirus.

PrimeScript One-Step RT-PCR Kit (Mountain View, CA) and spike gene-specific primers were used for PCR. The PCR products were sent for Sanger sequencing by the Genomics Technology Core at the University of Missouri-Columbia.

Cytokine and Chemokine Expression
Total RNA was transcribed to complementary deoxyribonucleic acid (cDNA) using SuperScript III Reverse Transcriptase with Oligo (dT)20. The qPCR amplification mixture contains the following: 7 μL water, 10 μL PowerUp SYBR Green Master Mix, 1 μL of each forward and reverse primers (10 μM), and 1 μL cDNA. The parameters of the qPCR were as follows: 1 cycle at 50°C for 2 minutes, 1 cycle at 95°C for 2 minutes, followed by 40 cycles at 95°C for 1 second and 60°C for 30 seconds. The relative mean fold changes for genes of interest are normalized by 40 cycles at 95°C for 1 second and 60°C for 30 seconds.

Microneutralization Assay
Sera were heat inactivated for 60 minutes at 56°C. The Dulbecco’s modified Eagle’s medium high glucose supplemented with 2% fetal bovine serum and antibiotic-antimycotic was used as diluent. Two-fold serial dilutions of the serum, starting from 1:10, were mixed with an equal volume of virus with 100 TCID₅₀ (50% tissue culture infective dose) of SARS-CoV-2. The serum-virus mixture was incubated for 1 hour at 37°C. After incubation, Vero E6 cells was added into each well. The cytopathogenic effect was observed for neutralization titers after a 72-hour incubation at 37°C with 5% CO₂.

RESULTS
A 25-year-old pregnant multigravida at 27 + 5 weeks gestation age, with diet-controlled gestational diabetes and a body mass index of 17, presented to the emergency department (ED) with mild COVID-19 symptoms, including 103°F fever, scratchy throat, and cough. The patient was diagnosed with SARS-CoV-2 infection, with reassuring fetal monitoring in the ED. Three days after diagnosis, the patient presented with vaginal bleeding and decreased fetal movement, went into active labor, and delivered a demise male infant vaginally. The patient’s postpartum course was complicated by disseminated intravascular coagulation, and she was discharged home after 2 days of hospitalization.

As per routine IUFD evaluation, the patient was IgM negative but IgG positive against parvovirus B19 and cytomegalovirus. She was immune to rubella and negative for toxoplasma. Immunohistochemistry (IHC) was negative in placenta for cytomegalovirus and herpes simplex virus. Her urine toxicology screen was negative for controlled substances. The fetus did not display any gross anomalies. Fetal chromosomal microarray confirmed a normal male with no clinically relevant copy number changes or loss of heterozygosity. The patient declined an autopsy for further investigation. These results suggested most likely cause of IUFD did not include infections, chromosomal abnormalities, or harmful drugs.

Viral RNA from the placenta was COVID-19 positive. The virus was determined to be a Delta variant by sequencing, with the spike genetic mutation signature including T19R, R158G, L452R, T478K, D614G, P681R, and D950N, and deletions at positions 156 and 157 compared with the prototype pandemic Wuhan strain. Currently, 2 Delta variants (Delta-142G and Delta-142D) are cocirculating in the United States, and this patient was infected with Delta-142D, which has been predominant in Missouri since May 2021.

Pathological analysis of the placenta was performed. The umbilical cord was slightly twisted and torn near the insertion site. There was no sign of maternal vascular malperfusion, viral inclusions, erythroblastosis, villous edema, suspicious lesions on the placental disk, membrane inflammation, or umbilical cord phlebitis or arteritis. However, pathology confirmed intervillos inflammatory composition of neutrophils and monocytes. The placenta showed villi with rims of fibrin, areas of degeneration, and loss of syncytiotrophoblastic layers. Syncytiotrophoblastic knots and stromal vascular karyorrhexis was seen (Figure 1A). This was observed in all examined sections, indicating diffuse involvement.

To understand the association of SARS-CoV-2 infection with IUFD, (1) IHC and qPCR were performed to confirm antigen presentation and (2) expression of inflammatory mediators in the placenta was measured. Extensive SARS-CoV-2 antigen was identified throughout the placental disc in villous cytotrophoblasts and throughout the cytoplasm of syncytiotrophoblasts (Figure 1B). In addition, abundant SARS-CoV-2 RNA was detected. Thus, we interpret this case as a Delta variant inducing an acute inflammatory response. Gene expression level of proinflammatory markers associated with viral
infection in the placenta were quantified. Compared with those markers in the placenta from a healthy woman, 9 of 13 dramatically increased from COVID-19 infection including tumor necrosis factor (TNF)-α with an increase of 199.60 (±29.02, standard deviation; \( P = .0329 \))-fold, interferon gamma-induced protein 10 (IP-10) (80.38 ± 27.21; \( P < .0001 \)), interferon (IFN)-α (54.97 ± 2.33; \( P < .0001 \)), interleukin (IL)-6 (19.38 ± 8.25), IFN-γ (16.96 ± 7.80), IL-8 (13.41 ± 3.03; \( P = .0016 \)), monocyte chemoattractant protein-1 (CCL2) (11.86 ± 0.24; \( P < .0001 \)), IL-10 (9.55 ± 4.41), and granulocyte colony-stimulating factor (G-CSF) (4.34 ± 1.93) (Figure 2). This response suggested that high levels of these proinflammatory markers stimulated by the Delta variant were associated with the trophoblastic necrosis and lesions crossing the placenta, which ultimately led to placental abruption.

Neutralizing antibody titers typically peak approximately 60 days after illness onset of mild COVID-19 infections \[9\]. Neutralizing antibody titers against both Delta variants from

**Figure 1.** Placental histopathology. (A) Hematoxylin and eosin staining of placenta sections from the patient and a healthy control. Control placenta is with intact syncytiotrophoblasts on the outer villous surface and no inflammation. The placenta of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) patient showed intervillous inflammation composed of neutrophils and monocytes (top right); villi with rim of fibrin and areas of degenerating and loss of syncytiotrophoblastic layer (bottom left); and villi with syncytiotrophoblastic knots and stromal vascular karyorrhexis (bottom right). (B) The SARS-CoV-2 spike protein-specific immunostaining of placenta sections from the patient and a healthy woman. The presence of SARS-CoV-2 antigens were identified in villous cytotrophoblast cells and cytoplasm of syncytiotrophoblast cells throughout the placenta (brown staining). The placenta from the healthy woman was collected before the emergence of the coronavirus disease 2019 (COVID-19) pandemic in the United States.
the patient's sera 2-months after disease onset were quantified. Results showed that the patient had homologous geometric mean titers of 320.00 ± 0.00 against Delta-142D and heterologous geometric mean titers of 201.59 ± 75.42 against Delta-142G. In comparison, the neutralizing titers against the early US variant (WA1) were still present but considerably less, with titers of 50.40 ± 18.86. The high neutralizing antibody level of the patient indicated stimulation of the immune response against SARS-CoV-2, suggesting that the patient was not immunocompromised and demonstrated a proinflammatory response to the pathogen.

**DISCUSSION**

The placenta is a physical and immunological barrier against fetal infection. Viral infection of the placenta triggers immune responses at the maternal-fetal interface. A cytokine storm at the placenta may trigger placental malperfusion and even abortion, and the intravascular coagulation at the utero-placental level can cause IUFD. The human placenta expresses receptors of SARS-CoV-2 and proteases that trigger viral fusion [10]. Pregnant women infected with SARS-CoV-2 during the first and third trimesters may have higher risks of inducing exaggerated responses causing a cytokine storm [11]. In the third trimester IUFD present in this study, our data suggest that the excessive infiltration of immune cells and cytokine storm at the placenta due to the Delta variant caused severe placental inflammation and damage, which likely resulted in placental abortion and the demise of the fetus. The limitation of our study is that we did not have access to control placental tissue from a gestational age-matched pregnant individual with gestational diabetes but no SARS-CoV-2 infection for comparison of the pathological and cytokine expression levels. Thus, our comparison was to a normal placenta. However, a previous study reported no significant differences in the expression of TNF-α, IL-6, IL-8, and several other genes of interest in the placentas of women with gestational diabetes mellitus relative to those of healthy pregnant women [12], whereas we found TNF-α and IL-8 were significantly elevated in our COVID-19 patient.

Studies showed that symptomatic pregnant women with COVID-19 are at increased risk for severe COVID-19-associated illness compared with nonpregnant women [1, 13]. During the third trimester, SARS-CoV-2-positive women were shown to have more maternal and fetal vascular malperfusion, villous agglutination, and subchorionic thrombi [14]. The presence of SARS-CoV-2 infection in placenta has also been reported in COVID-19 patients [3]. Among these cases of placental infections, one mother experienced a complicated second trimester pregnancy with preeclampsia and placental abruption, one had mild COVID-19 symptoms with a normal, healthy birth, and one experienced moderate symptoms while the neonate also contracted COVID-19 and suffered from neurological manifestations. However, IUFD associated with maternal SARS-CoV-2 infections have been generally rare, and all of these cases were associated with severe maternal complications [15–17]. The maternal characteristics and symptoms included overweight or obese individuals experiencing mild to moderate COVID-19 symptoms. In addition, different extents of placental inflammation were reported, such as inflammation and fibrin deposition in and between the villi [15], chorionitis, infarction, and necrosis of the placental parenchyma [16] and mixed inflammatory infiltrates in the subchorial space [17].

The histopathological changes of placenta in patients with diabetes were varied and inconsistent. The most frequent placental changes were represented by the immaturity of chorionic villus characterized by a high villus density with increased villus lumens and presence of syncytiotrophoblast nodules. Less frequent changes include the presence of stromal edema...
in the terminal villus, the presence of collagen fiber densifications in villus trunks >1 mm, and diffuse calcifications in the villus stroma. There is also evidence of minimal to no placental changes in some diabetic women compared with control groups [18].

This report adds to the literature and illustrates a case of IUFD in a pregnant woman caused by Delta variant. Evidence suggests that risk for stillbirth was significantly higher during the period of Delta predominance than during the pre-Delta period [5]. The rapid fetal demise indicates that close fetal monitoring of infected pregnant women, regardless of symptom severity, should be considered.

CONCLUSIONS

Coronavirus disease 2019 vaccines are widely accepted to be safe and stimulate robust antibody responses in pregnant women [19–23]. However, among pregnant women, there is only 24% vaccine coverage of individuals with at least 1 dose in the United States as of November 27, 2021. The rise of the Delta variant coupled with high vaccine hesitancy in pregnant women raises public health concerns regarding the potential for greater disease severity and worse outcomes, as observed in the unvaccinated patient discussed in this case. Evidence shows that the COVID-19 vaccine has high levels of effectiveness against symptomatic disease with the Delta variant after full vaccination [6, 24]. Thus, vaccination should continue to be strongly advocated and will likely continue to reduce SARS-CoV-2 infection risks for pregnant women and their fetuses.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the author. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank Rebecca Patterson, George Sarafianos, Naser Ashiekh, and Jess Bottger for their efforts in sample collection and Dr. Yang Wang for designing primers for sequencing. We appreciate routine assistance in the laboratory from Liping Long, Olivia Jacobson, and Haley Hudson. We are grateful for the facility and technical support from Jeffery Adamovicz, Travis McCarthy, and Paul Anderson at University of Missouri-Columbia.

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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