SHORT COMMUNICATION

Probable viremia and positive placental swabs for SARS-CoV-2 in a preterm pregnant woman with mild COVID-19

Yesim Akdemir | Demet Haciseyitoglu | Guven Celebi | Cumhur Aydemir | Burak Bahadir | Anil T. Cakir | Aykut Barut | Ulku Ozmen

1Department of Obstetrics and Gynaecology, School of Medicine, Zonguldak Bulent Ecevit University, Zonguldak, Turkey
2Department of Microbiology, School of Medicine, Zonguldak Bulent Ecevit University, Zonguldak, Turkey
3Department of Infectious Diseases and Clinical Microbiology, School of Medicine, Zonguldak Bulent Ecevit University, Zonguldak, Turkey
4Department of Pediatrics and Neonatal Critical Care, School of Medicine, Zonguldak Bulent Ecevit University, Zonguldak, Turkey
5Department of Pathology, School of Medicine, Zonguldak Bulent Ecevit University, Zonguldak, Turkey

Correspondence
Yesim Akdemir, Department of Obstetrics and Gynaecology, School of Medicine, Zonguldak Bulent Ecevit University, Zonguldak 67100, Turkey.
Email: yesimakdemir@yahoo.com

Abstract
This study aimed to report a case of mild novel coronavirus disease (COVID-19) in a pregnant woman with probable viremia, as reverse transcription-polymerase chain reaction (RT-PCR) testing of endometrial and placental swabs for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was positive. A 26-year-old multigravida at 35 weeks 2 days of gestation, who had extensive thigh and abdominal cellulitis, tested SARS-CoV-2 positive by RT-PCR performed on samples from the endometrium and maternal side of the placenta. However, other samples (amniotic fluid, fetal side of the placenta, umbilical cord, maternal vagina, and neonatal nasopharynx) tested negative for SARS-CoV-2. This is one of the rare reports of probable SARS-CoV-2 viremia with the presence of SARS-CoV-2 in the endometrium and placenta, but not leading to vertical transmission and neonatal infection. Because knowledge about transplacental transmission and results is very limited, we conclude that more RT-PCR tests on placental and cord blood samples are needed in order to safely make definite conclusions.

KEYWORDS
COVID-19, placenta, pregnancy, SARS-CoV-2, vertical transmission

1 | INTRODUCTION

Since the novel coronavirus disease (COVID-19) outbreak, which is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), became a pandemic, numerous papers have reported pregnant women with both mild and severe COVID-19.1–4 Although early reports with limited numbers of patients showed that most pregnant women with COVID-19 had mild disease,5 increased hospitalization rate (RR: 5.4), need for intensive care unit admission (RR: 1.5), and need for invasive mechanical ventilation (RR: 1.7) were found in the study conducted by the Centers for Disease Control and Prevention, which included 8209 pregnant women with COVID-19.5 Today, the effects of the disease on pregnancy are not completely understood.

One other important and the possible issue is vertical transmission, but data is scarce. Angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2) are essential for the fusion and entry of SARS-CoV-2 into host cells. Meanwhile, it was shown that ACE2 and TMPRSS2 expressions are present and increase from the first to the second trimester in maternal-fetal interface cells and multiple fetal organs.6

Several case reports and series reported suspected cases of vertical transmission by testing for SARS-CoV-2 using placenta, amniotic fluid, maternal vaginal swab, neonatal cord blood, and neonatal nasopharyngeal and rectal swab samples or testing for specific neonatal antibodies,7–16 whereas others could not.17–23 Regardless of the concerns about the quality of evidence, the optimal definition of vertical transmission was not clear. Recently, Blumberg et al.24 suggested a
classification for SARS-CoV-2 vertical transmission as follows: intrauterine transmission, intrapartum or early postnatal transmission, and superficial exposure to SARS-CoV-2 or transient viremia.

Herein, we report a case of mild COVID-19 in a pregnant woman with probable viremia, as reverse transcription-polymerase chain reaction (RT-PCR) testing of endometrial and placental swabs for SARS-CoV-2 were positive. Institutional review board approval was obtained and patient information was obtained from medical records.

2  |  CASE REPORT

A 26-year-old woman, Gravida 2 Para 1, at 35 weeks and 2 days of gestation was transferred to our clinic on August 31, 2020, due to abdominal pain and pruritus. She was morbidly obese, with a body mass index of 37.47. She did not receive proper antenatal care due to her fears about the COVID-19 outbreak. She reported that her first-trimester aneuploidy screening test was within the normal risk limits, but no second-trimester fetal ultrasound scan or oral glucose tolerance test was performed. Her fasting and postprandial blood glucose levels were normal, even though HbA1c was 6.2%. Her blood type was AB Rh (–) and there was no history of Rh sensitization during her current or previous pregnancies. She had a history of cough, malaise, and fatigue, which started 5 days before admission, and worsened with dyspnea and fever 1 day before admission. She had warm and reddish abdominal lesions below the umbilicus and bilateral posterior thigh pain, which had started 1 month prior. At admission, the temperature was 36.1°C, blood pressure was 105/80 mmHg, pulse rate was 92/min, respiratory rate was 20/min, and blood oxygen saturation was 98% in room air. She also had mild dyspnea and cough.

Obstetric examination revealed a macrosomic fetus (estimated fetal weight was 3548 g, 37 2/7 gestational weeks' average, >97th percentile) with normal levels of amniotic fluid. Nonstress test was applied with WHO guidance.25 Before nucleic acid purification, whole blood samples according to the manufacturer’s recommendations. About 400 μl of all samples were added to EZ1 DSP Cards of the Qiagen EZ1 Advanced XL system (Qiagen). All samples were extracted using the Qiagen EZ1 Advanced XL system (Qiagen) to automatically extract sample RNA. The detection of SARS-CoV-2 RNA was performed with SARS-Cov-2-Divine Gene RT-qPCR Amplification Kit that targeted the ORFlab and N genes of SARS-CoV-2 (BioSpeedy, Bioeksen). The lower detection limit (LoD) reported by the Ministry of Health’s General Directorate of Public Health was 200 genomes/ml, analytical sensitivity and specificity were 99.4% and 99.0%, respectively. Real-time RT-qPCR was performed using Rotor-Gene 5r Plex Real-Time PCR Systems (Qiagen). A cycle threshold value (Ct-value) less than 38 was defined as a positive test result, and a Ct-value of 38 or more was defined as a negative test result.

One day after admission, she had regular uterine contractions with cervical effacement and dilatation of 1 cm. Emergency cesarean section was performed with maximal caution and protection because she had a history of previous cesarean birth. The patient wore a surgical mask throughout the cesarean section. The amniotic fluid sample was obtained via direct syringe aspiration before rupturing the amniotic membrane, and a male newborn weighing 4040 g was delivered, with Apgar scores of 7 and 8 at 1 and 5 min, respectively. The cord was clamped without delay, and skin-to-skin contact was not permitted. Umbilical cord blood samples were collected by direct syringe aspiration after delivery. The fetal side of the placental swab was obtained from the amniotic surface near the umbilical cord after clearing the maternal blood. The maternal side of the placental swab was obtained inside of the nearest cotyledon in the level of the umbilical cord. Endometrium inner surface swabs were performed from all uterine walls and fundus. The placenta was sent to the pathology department for a detailed microscopic examination. The newborn was immediately transferred to the neonatal care unit and a neonatal nasopharyngeal swab was obtained via FLOQSwabs at birth to ascertain the possibility of intrauterine fetal infection, and other throat swab samples were taken on the day of life 1 and on day of life 4. In addition, samples of maternal vaginal secretions and breast milk were obtained after delivery.

Amniotic fluid, maternal and fetal side of the placenta, umbilical cord blood, vaginal secretion, and endometrium inner surface samples were obtained via FLOQSwabs (COPAN Diagnostics Inc) at the time of delivery in the operating room. All samples placed in a UTM-RT medium for viruses (COPAN) were sent to our microbiology laboratory in half an hour. Sample collection was performed by trained physicians, transport to the laboratory was carried out at 4°C with transport containers. Samples were stored at 4°C till testing (around 30 min). Sample collection, processing, and laboratory testing complied with WHO guidance.25 Before nucleic acid purification, whole cord blood was added to buffer ATL (Qiagen products, Germany) to a final volume of 400 μl (200/200) for pretreatment of whole blood samples according to the manufacturer’s recommendations. Samples from the endometrium and maternal side of the placenta, umbilical cord, maternal vagina, and neonatal nasopharynx were tested
| Table 1 | Laboratory findings at follow-up |
|---------|----------------------------------|
|         | 08/31 | 09/01 | 09/02 | 09/04 | 09/06 | 09/09 | 09/25 | Reference range |
| WBC (x10^3) | 8.4 | 9.7 | 9.6 | 9.3 | 7.5 | 3.9 | 7.5 | 3.6–10.2 |
| Platelet (x10^3) | 154 | 158 | 174 | 265 | 303 | 424 | 340 | 152–348 |
| Hemoglobin (g/dl) | 10.4 | 12.5 | 12.5 | 11.4 | 10.4 | 10.2 | 13.1 | 12.5–16.3 |
| Lymphocyte (x10^3) | 0.5 | 0.4 | 0.4 | 0.5 | 0.5 | 0.5 | 0.9 | 1–3.2 |
| CRP (mg/dl) | 97.4 | 109.8 | 173.0 | 201.6 | 131.6 | 33.5 | 21.91 | 0.00–5.00 |
| D-dimer (µg/ml) | 4.02 | 3.76 | 2.68 | 3.71 | 3.60 | 6.85 | 1.07 | 0.00–0.50 |
| Ferritin (mg/dl) | 121.7 | 83.7 | 123.3 | 129.0 | 267.9 | 162.6 | – | 13.0–150.0 |
| Fibrinogen (mg/dl) | 362.0 | 389.0 | 341.0 | 567 | 602 | 445 | 454 | 193.0–412.0 |
| Procalcitonin (ng/ml) | 1.54 | 1.87 | 49.39 | 24.05 | 3.81 | 0.79 | – | 0.00–0.05 |
| Troponin T (ng/ml) | 0.005 | 0.007 | 0.007 | 0.007 | 0.003 | 0.004 | 0.003 | 0.00–0.03 |
| Glucose (mg/dl) | 75.0 | 84.0 | – | – | – | 90.0 | – | 70.0–110.0 |
| HbA1c (%) | 6.2 | – | – | – | – | – | – | 4.0–5.9 |
| AST (U/L) | 248.0 | 222.0 | 114.0 | 21.0 | 46.0 | 38.0 | 22.0 | 0.0–32.0 |
| ALT (U/L) | 134.0 | 150.0 | 95.0 | 60.0 | 23.0 | 38.0 | – | 0.0–33.0 |
| Creatinine (mg/dl) | 0.8 | 1.2 | 1.4 | 0.9 | 0.5 | 0.4 | 0.6 | 0.0–1.2 |
| BUN (mg/dl) | 21.0 | 30.0 | 29.0 | 26.0 | 19.0 | 18.0 | 23.0 | 14.0–50.0 |
| Uric acid (mg/dl) | 6.9 | 8.8 | 8.8 | 9.5 | 3.4 | 2.6 | – | 2.4–5.70 |
| LDH (U/L) | 530.0 | 374.0 | 477.0 | 320.0 | 265.0 | 312.0 | – | 135.0–241.0 |
| Total bilirubin (mg/dl) | 0.92 | 1.71 | 1.27 | 0.55 | 0.40 | 0.36 | 0.62 | 0.0–1.2 |
| Direct bilirubin (mg/dl) | – | 1.57 | 1.10 | 0.42 | 0.30 | 0.20 | – | 0.00–0.04 |
| PT (s) | 9.13 | 9.11 | 9.34 | 9.33 | 8.35 | 9.14 | – | 8.4–10.6 |
| INR | 1.01 | 1.00 | 1.03 | 1.03 | 0.99 | 1.01 | – | 0.93–1.17 |
| aPTT (s) | 39.5 | 40.4 | 32.0 | 37.7 | 34.7 | 32.9 | – | 23.9–33.20 |
| Creatine kinase (U/L) | 56.0 | 57.0 | 199.0 | 125.0 | 43.0 | 53.0 | – | 0.0–170.0 |

Blood gas analyze

| pH | 7.45 | – | – | – | – | – | – | 7.35–7.45 |
| pCO2 (mmHg) | 22.3 | – | – | – | – | – | – | 32.0–48.0 |
| pO2 (mmHg) | 80.8 | – | – | – | – | – | – | 83.0–108.0 |
| BE (mmol/L) | –6.9 | – | – | – | – | – | – | –2.0 to 2.0 |
| HCO3 (mmHg) | 18.8 | – | – | – | – | – | – | 22.0–28.0 |

aWhite blood cell.

bC-reactive protein.

cAspartate aminotransferase.

dAlanine aminotransferase.

fBlood urea nitrogen.

gLactate dehydrogenase.

hProthrombin time.

iActivated partial thromboplastin time.
negative for SARS-CoV-2. Histopathological examination of the placenta showed neither inflammation nor vasculopathy (Figure 2).

After delivery, she was put on favipiravir treatment for 5 days. The mild dyspnea and cough disappeared in 2 days and the blood oxygen saturation remained normal (98%–99%). She was afebrile with normal vital and puerperal signs for 8 days until discharge. However, the abdominal cellulitis area tended to expand and CRP levels increased to 232.1 mg/dl on postoperative day 2. Teicoplanin was added to the meropenem therapy. Inflammatory markers and liver enzymes gradually normalized (Table 1) and the cellulitis resolved. A second SARS-CoV-2 RT-PCR test was positive on September 9, 2020. She was discharged on postoperative day 8 and advised to follow home quarantine rules. The third SARS-CoV-2 RT-PCR test was planned after 14 days and came out negative.

After the newborn was transferred to the neonatal intensive care unit due to the transient tachypnea of the newborn, he was isolated and nasal continuous positive airway pressure (NCPAP) was used to reduce respiratory distress. He was successfully weaned from NCPAP after 24 h and received free-flow oxygen therapy in an incubator for three additional days. Breastfeeding was discouraged and he was fed with formula. His blood type was B Rh (+), the Coombs test was positive, and hyperbilirubinemia was detected, for which intensive phototherapy and intravenous immunoglobulin treatment were administered. Nasopharyngeal SARS-CoV-2 RNA RT-PCR test on Day 1 of life and repeat test on Day 4 of life were all negative. The newborn was discharged 10 days after birth.

3 | DISCUSSION

We report the case of a pregnant woman with mild COVID-19 and possible SARS-CoV-2 viremia but not leading to vertical transmission and neonatal infection.

Although uncommon, viremia could occur after the incubation period; Wang et al.26 found SARS-CoV-2 particles in 1% of blood samples. SARS-CoV-2 spike glycoprotein along with viral entry proteins ACE2 and TMPRSS2 were shown in the villous compartment of the placentas of pregnant women with COVID-19.8 These findings could easily justify vertical transmission by the transplacental route, but COVID-19 infection has not been detected in all neonates born of infected pregnant women.

One study found elevated IgG and IgM antibodies in 2-h newborns, but neonatal throat swab was negative.15 Another study found that 50% of newborns (3/6) had elevated IgM antibodies, but all neonatal throat swabs were negative.14 It is already known that IgG transfer begins as early as 13 weeks of gestation and the rate of transfer increases after the second trimester, but the IgM macromolecule unlikely crosses the placenta.27 Sensitivity and specificity of SARS-CoV-2 IgM immunoassays were reported to be 48.1% and 100%, respectively; thus, the results should be interpreted cautiously.28 Inflammation or injury could lead to a disruption in the integrity of the maternal-fetal interface and leakage of maternal IgM antibodies into the fetal circulation. On the other hand, Zarnamian et al.29 found a positive result with neonatal throat swab and amniotic fluid sample in a preterm pregnant woman with severe COVID-19. Another study reported a positive nasopharyngeal swab result in a 16-h newborn with confirmation 48 h later.24

The transplacental transmission was highly suggested by Vivanti et al.,7 in their case of a pregnant woman with mild COVID-19 case, as E and S genes of SARS-CoV-2 were found in maternal blood, placenta, amniotic fluid, vaginal swab, neonatal blood, neonatal rectal swab, and three repeated neonatal nasopharyngeal swab samples. In a severe case of COVID-19, both maternal blood sample and neonatal throat, blood, and feces samples were positive.10 Patane et al.9 described two cases in which the mother, fetal side of the placenta, and the neonates were positive for SARS-CoV-2 out of 22 cases. SARS-CoV-2 spike glycoprotein was detected in placenta in which chronic intervillositis was shown along with placental macrophages, both in the intervillous and villous spaces. A similar case was
reported by Kirstman et al., in which the mother, both the fetal and maternal sides of the placenta, and repeated neonatal nasopharyngeal tests were positive for SARS-CoV-2, and the placenta showed multiple areas of histiocytic intervillitis and infarction. In an interesting case of a woman with COVID-19 in the second trimester who presented with severe hypertension, placental abruption, and an abortion, placenta and umbilical cord blood were positive for SARS-CoV-2 and the placenta showed intervillitis with SARS-CoV-2 spike glycoprotein, which was predominantly detected in syncytiotrophoblasts. Authors suggested that COVID-19 might have worsened the placental dysfunction, leading to early-onset pre-eclampsia and placental abruption.

The placenta is the main barrier against viral infections, as it has an organ-specific antiviral mechanism. Fused and periodically re-generated syncytiotrophoblasts have no gap junctions and form a tight physical barrier. Immune defense systems are activated by the type III IFN signaling, autophagy triggering trophoblastic miRNAs, and the NF-KB pathway. The core of the placental villi is enriched in mesenchymal stem cells, which can differentiate into multiple cell lineages (dNK cells, macrophages, T regulatory cells, and Hofbauer cells) responsible for immunomodulation and anti-inflammation, thus creating an immunomodulatory defense. Altogether, placental functions could protect the fetus; however, there may be cases where the placenta per se is insufficient. The defense layer formed by the syncytiotrophoblasts may be deficient in the first and early second trimesters due to improper lining or in late pregnancy, when syncytium formation starts to decline. In addition, maternal systemic disorders or inflammatory and vascular changes could cause hypoxic or immune injury in the placenta, which further disrupts the placental integrity.

The relationship between viral load and disease severity, mortality, comorbidities (congestive heart failure, diabetes, and chronic kidney disease), and increasing age has been shown in adults. However, the relationship between SARS-CoV-2 viral load and vertical transmission is not fully understood. Demirjian et al. suggested that high pulmonary viral load might increase maternal viremia and facilitate vertical transmission, as they detected SARS-CoV-2 in neonatal blood, neonatal upper respiratory tract, and feces in a case of a pregnant woman with severe COVID-19 who required extracorporeal membrane oxygenation support. Therefore, the placental viral load might be an important factor that must be addressed. Placental viral load was much higher than maternal upper respiratory tract, vaginal, blood, and amniotic fluid viral load in a woman in late pregnancy with COVID-19 and suspected SARS-CoV-2 vertical transmission.

We detected SARS-CoV-2 in the endometrium and maternal side of the placenta in a preterm pregnant woman with mild COVID-19. There was no vertical transmission. Samples from the fetal side of the placenta and amniotic fluid, neonatal cord blood, neonatal nasopharynx, and breast milk samples were all negative. Other studies found similar findings along with our study. Hsu et al. reported placental SARS-CoV-2 with placental vasculopathy in a term pregnant woman with mild COVID-19 without vertical transmission. Ferraiolo et al. found positive placental swab for SARS-CoV-2 in a term pregnant woman with asymptomatic COVID-19. Another study found SARS-CoV-2 RNA in placental or membrane swabs in 3 out of 11 placentas without any evidence of vertical transmission and neonatal infection.

Even though we did not show viremia in maternal blood, we hypothesize that while being rare, maternal viremia might lead to placental infection in some circumstances, which could facilitate the placental dysfunction mentioned earlier. When the physical and immunological barrier function of the placenta is disrupted, congenital infections could occur. However, there are still important unanswered questions. Firstly, which pregnant population is susceptible to viremia and placental infection; secondly, is COVID-19 severity and/or viral load related to vertical transmission, and finally, why do all placental infections not always result in congenital or neonatal infections.

Our report has some limitations. Although contamination is unlikely, as all other swabs except endometrium and maternal side of the placenta, were negative for SARS-CoV-2 and we had multiple samples of SARS-CoV-2 from multiple different sites to avoid false positivity of the RT-PCR assay, swabs were not repeated except for maternal and neonatal nasopharynx. Immunostaining is not available for SARS-CoV-2 in our Pathology Department; therefore, we could not identify the SARS-CoV-2 gene targets that most of the studies reported. Nevertheless, this is one of the rare reports of probable viremia and placental infection of SARS-CoV-2. More evidence on placental SARS-CoV-2 RNA increases fears about congenital infections, that its effects would be in a spectrum between maternal immune activation-related neonatal disorders and congenital anomalies or syndromes. Further investigations that focus on the short- and long-term effects of SARS-CoV-2 infection on neonates in different trimesters of pregnancy are needed.

**CONFLICT OF INTERESTS**

The authors declare that there are no conflict of interests.

**AUTHOR CONTRIBUTIONS**

Yesim Akdemir and Demet Haciseyitoglu were responsible for the organization and coordination of the trial. Yesim Akdemir was the chief investigator and was responsible for the data analysis. Cumhur Aydemir, Burak Bahadir, and Guven Celebi developed the trial design. All authors contributed to the writing of the final manuscript.

**ORCID**

Yesim Akdemir https://orcid.org/0000-0002-8574-5065

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