Case Report

Gamma variant vertically transmitted from a mild symptomatic pregnant woman associated with fatal neonatal COVID

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ARTICLE INFO

Article history:
Received 3 March 2022
Accepted 7 June 2022
Available online 11 July 2022

Keywords:
Sars-CoV-2 variant
Vertical transmission
Covid 19
Preterm infant

ABSTRACT

Herein we describe a mild symptomatic real-time reverse transcriptase-polymerase chain reaction-confirmed coronavirus 2 (SARS-CoV-2) infection in a pregnant woman who gave birth to a preterm infant, 32 weeks gestational age. The neonate was immediately isolated after delivery and developed severe respiratory disease that progressed to multisystem inflammatory syndrome and death on the seventh day of life. Genome sequencing detected the P.1 (gamma) variant in samples obtained at hospital admission (mother) and on the first (10h) and 13th days of life (neonate). Complete homology (mother’s and newborn’s sequences) confirmed vertical transmission. To our knowledge, this is the first report of vertically-transmitted SARS-CoV-2 P.1 (gamma) variant in a mild symptomatic infection in pregnancy associated with fatal COVID in a neonate.

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Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) P.1 (gamma) is a variant that has circulated in Brazil. It was first detected in Amazonas state in late 20201 and subsequently spread throughout Brazil. By May 2021, the coronavirus disease (COVID-19) pandemic in Brazil was at its worst, resulting in thousands of deaths.2 Available data on the P.1 (gamma) variant have suggested an increased transmissibility and a higher risk of reinfection than non-P.1 variants.1,3 However, its role in vertical transmission remains unknown.

Herein we report a case of SARS-CoV-2, P.1 (gamma) variant vertical transmission to a premature baby born to a mother with mild symptomatic COVID-19. The neonate developed severe respiratory disease that progressed to multisystem inflammatory syndrome (MIS-C) and death.

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https://doi.org/10.1016/j.bjid.2022.102385
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Case report

A previously healthy 30-year-old pregnant woman (gravida 3, para 1, fetal loss 1) was admitted at Children Hospital of Ribeirão Preto Medical School in late March 2021 at 31 weeks and 4 days of gestation (calculated using ultrasound at 12 weeks) because of premature rupture of membranes. Considering her 3-day history of ageusia and anosmia, SARS-CoV-2 real-time reverse transcriptase polymerase chain reaction (RT-PCR) was performed on oral and nasopharyngeal swabs obtained at hospital admission, yielding positive results. The patient remained clinically stable without any respiratory symptoms. Her syphilis, HIV (human immunodeficiency virus), and toxoplasmosis serology screening in the first trimester and findings of vaginal-rectal cultures for Group B Streptococcus obtained two days before delivery were negative. Upon admission, her C-reactive protein (CRP) levels and complete blood count (CBC) were normal. She received two doses of betamethasone (6 mg/day) and enoxaparin (60 mg/day).

After remaining stable for four days, she developed fever (38°C) with a shift to the left on CBC [6.1 × 10³/μL leukocytes (2% promyelocytes, 1% myelocytes, 4.2% bands)] and a high CRP level [7.2 mg/dL (normal value < 1.0)]. She was diagnosed with chorioamnionitis; thus, antibiotic treatment with clindamycin was initiated. At the onset of her spontaneous labor in breech presentation, cesarean delivery was performed in a negative pressure room under epidural anesthesia. The mother and staff wore correct personal protective equipment.

Delayed cord clamping, skin-to-skin contact, and breastfeeding were avoided, and contact between the neonate and mother after delivery was not initiated. The mother recovered her good clinical conditions, remained asymptomatic, and was discharged 3 days after delivery (Table 1).

The male infant weighed 2,160 g (adequate for gestational age) with 8/9 Apgar scores. He was immediately transferred to a separate room and did not need resuscitative measures. Non-invasive continuous positive airway pressure was initiated within 10 minutes of life due to mild respiratory distress. The neonate was transferred to a negative pressure isolation room in the neonatal intensive care unit (NICU). At two hours after remaining stable for four days, she developed fever (38°C) with a shift to the left on CBC [6.1 × 10³/μL leukocytes (2% promyelocytes, 1% myelocytes, 4.2% bands)] and a high CRP level [7.2 mg/dL (normal value < 1.0)]. She was diagnosed with chorioamnionitis; thus, antibiotic treatment with clindamycin was initiated. At the onset of her spontaneous labor in breech presentation, cesarean delivery was performed in a negative pressure room under epidural anesthesia. The mother and staff wore correct personal protective equipment.

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Sequencing of the neonate showed three other mutations in the spike (S) protein (R152C, T478I, Q493R) that were not present in the reference sequences, indicating that he was suffering from active COVID-19.

Laboratory techniques

Oral and nasopharyngeal swabs were collected upon the mother’s hospital admission and neonate’s first (10 hours) and 13th DOL. SARS-CoV-2 RNA was detected from 100 μL of nasopharyngeal swab suspension. RNA extraction was performed using the Extracta Kit FAST DNA e RNA Viral (Loccus, SP, Brazil) in an automated extractor (EXTRACTA 32; Loccus) following the manufacturer’s guidelines. SARS-CoV-2 RT-PCR was performed using the Gene FinderTM COVID19 Plus RealAmp kit (OSang Healthcare Co. Ltd.), which detects RdRp, E, and N genes. The reaction protocol was performed according to the manufacturer’s protocol using the 7500 Real-Time PCR System (Thermo Fisher Scientific).
| Clinical data | Maternal evolution | Neonatal evolution |
|---------------|-------------------|-------------------|
| 31 weeks and 4 days | 31 weeks and 6 days | 32 weeks and 1 day | 32 weeks and 3 days | 3 days after delivery | 1-21 Days | 22-29 days | 30-36 days | 37-74 days |
| Diagnosis | - | Premature rupture of membranes | Chorioamnionitis | Spontaneous labor in breech presentation | - | Neonatal respiratory distress syndrome | Septic shock | MIS-C | Death |
| Clinical data | Ageusia and anosmia | Ageusia and anosmia | Fever (38°C), Ageusia and anosmia | Ageusia and anosmia | - | Seizures Respiratory distress Shock; Cardiac arrest (10 min) | Pulmonary hypertension. (PAP: 60 mmHg) Shock. | MIS-C | Persistent hypoxemia and pulmonary hypertension Acute myocardial infarction Cardiac failure |
| Laboratory findings | Positive SARS-CoV-2 RT-PCR | Left shift on CBC [6.1 × 103/μL leukocytes (2% promyelocytes, 1% myelocytes, 42% bands)] and a high CRP level [7.2 mg/dL (normal value <1.0)] | Ferritin: 821.90 | Troponin: 1,175.00 | Troponin: 15,780.10 | Ferritin: 1515.6 NT-Pro-BNP (pg/mL) >35,000 |
| Treatment | Hospitalization | Two doses of betamethasone (6 mg/day) and enoxaparin (50 mg/day). | Clindamycin was initiated | Cesarean delivery | Discharge (domiciliary isolation) | Exogenous surfactant; Mechanical ventilation; Vasoactive drugs; Enoxaparin; Azithromycin; Hydrocortisone | iNO; Vasoactive drugs; | iNO; Vasoactive drugs; | iNO; Sildenafil; Bosentan; Exogenous surfactant; Metoprolol; Intravenous immunoglobulin n; High-dose pulse steroids (methylprednisolone) |

* Normal reference values: ferritin 22-322 ng/mL; NT-pro-BNP <125 pg/mL; PT 1.3 sec; troponin I <19 ng/dL.

Abbreviations: iNO, inhaled nitric oxide; NT-pro-BNP, N-terminal pro B-type natriuretic peptide; PAP, pulmonary artery pressure.
Sequencing

SARS-CoV-2 complete genomic sequences were obtained through Illumina COVIDSeq technology according to the manufacturer’s protocol. Sequencing libraries were pooled, normalized to 4 nM, and denatured with 0.2 N NaOH and 400 mM Tris-HCl (pH-8). Each sample library (9 pM) was loaded onto a 300-cycle MiSeq Nano Reagent Kit v2 and run on an Illumina MiSeq instrument (Illumina, San Diego, CA, USA).

Bioinformatic analysis

Raw sequence data were subjected to quality control analysis using FastaQC software version 0.11.8. Trimming was performed using Trimmomatic version 0.3.95 to select best quality sequences. Bioinformatic analyses were performed on sequences with quality scores > 30. We mapped the trimmed sequences against the SARS-CoV-2 reference (GenBank refseq NC_045512.2) using BWA (Burrows-Wheeler Aligner) software and samtools for read indexing. Mapped files were submitted to refinement with the Pilon software to obtain the most accurate information on indels and insertions. Afterwards, the trimmed sequences were subjected to a remap against the genome refined by Pilon. Finally, we used bcftools for variant calling and seqtk to create a consensus genome.

Phylogenetic analysis

A representative subset of 3,874 genomes obtained from GISAID was obtained following the Nextstrain guidelines. Two full-length novel genomes were appended to this subset for further analysis. Sequence alignment was performed using MAFFT v7.475 and manually curated to remove artifacts using Aliview. Maximum likelihood (ML) phylogenetic trees were estimated using IQtree v.16.12, applying the ML algorithm with statistical support of ultrafast bootstrap with 1000 replicates. The nucleotide substitution model was GTR+F, chosen according to the Bayesian information criterion statistical model. The final formatting and visualization of the phylogenetic tree were performed using the ggtree R package.

Mutational pattern analysis

Mutational profiles were investigated using the Nextclade tool to describe substitutions. Subsequently, the set of non-synonymous mutations was compared to the profiles available in the PANGO lineage resource to attribute genomes to lineages.

Sequencing information

The mother’s and newborn’s samples yielded 188,205 reads with a mean depth of 675,948 and 99.93% coverage and 154,023 reads with a mean depth of 593.93% and 99.92% coverage, respectively.

Ethics approval

The study was approved by the Research Ethics Committee of the Medicine School in Ribeirão Preto, University of São Paulo, Brazil (CAAE: 48798421.7.0000.5440 - 4.835.538/2021) and from the Brazilian hospitals and maternal services. The parents agreed and signed the consent to data publishing.

Discussion

To our knowledge, this is the first documented case of SARS-CoV-2 P.1 (gamma) variant vertical transmission, from mild symptomatic mother, associated with fatal COVID-19 in the neonate, starting with respiratory failure complicated by MIS-C and evolving to neonatal death.

Intrauterine fetal exposure to SARS-CoV-2 was confirmed by a positive RT-PCR from the neonate’s sample collected 10 hours after birth, suggesting mother-to-child transmission. This finding was not associated with direct contact between the mother and infant as they were separated immediately after delivery. Most important for the evaluation of SARS-CoV-2 vertical transmission is the finding of a complete homology between the mother’s and newborn’s sequences, evidencing their origin from a single source. Furthermore, the detection of SARS-CoV-2 on a sterile sample obtained from the neonate on the 13th DOL confirmed his ongoing infection.

Effects of SARS-CoV-2 infection in fetuses and newborn infants remain unknown. Recent systematic reviews on neonates born to SARS-CoV-2-infected mothers reported vertical transmission rates of 3.2%-4.2%, based on positive RT-PCRs using nasopharyngeal swabs obtained before 48 hours of life. However, RT-PCR, which is the gold standard diagnostic test, was not universally performed and was mostly obtained after 48 hours of life. Thus, the description of vertical transmission in the literature is frequently incomplete, hindering confirmation of this mode of transmission. Also, the report of vertical transmission in a mild COVID pregnant woman is scarce, leading to sub notification of mother-to-child SARS-CoV-2 transmission.

Tropism of SARS-CoV-2 to the fetus is a concern, as the angiotensin-converting enzyme 2 receptor used by the virus to invade cells, is found in placental cells and fetal tissues. Most reports of neonatal COVID-19 have notably described the presence of mild symptoms, with approximately 2% of newborns requiring NICU admission. The most commonly reported signs and symptoms were respiratory abnormalities (52.5%), fever (44.3%), and gastrointestinal (36%), neurological (18.6%), and hemodynamic manifestations (10.3%). However, MIS-C is a rare manifestation of SARS-CoV-2 in children, more common in older children, and neonates seem less affected. Only a few cases of MIS-C in newborns have been reported, although none had a definite association with vertical transmission. Since 2020, few case reports of SAR-CoV-2 vertical transmission have been described; in Table 2, we presented the case reports of newborns from mothers positive for coronavirus and her infants who presented SARS-CoV-2 RT-PCR positive, indicating vertical transmission. We only considered
infants in whom nasal RT-PCR was positive without previous contact with the mother. We showed seven cases, and only one identified the variant (Delta variant – B1) associated with the vertical transmission.21–27

This is the first case of SARS-CoV-2 P.1 (gamma) variant vertical transmission in a mild symptomatic mother, with confirmed mother-to-child transmission, followed by probably neonatal MIS-C and death. Considering that an association between the severity of neonatal disease and maternal P.1 (gamma) variant infection cannot be ruled out, this report raises concerns about the impact of infection by this specific variant on maternal - neonatal health.

## Conclusion

SARS-CoV-2 vertical transmission is a possible finding and can be associated with fatal neonatal COVID, even in neonates born to a mild symptomatic woman. In the face of the different variants that have emerged worldwide, further studies focusing on infection by different variants and neonatal outcomes are essential to understand better the repercussions of infection by distinct SARS-CoV-2 variants in pregnant women and their newborns.

## Conflicts of interest

The authors declare no conflicts of interest.

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