Placental maternal vascular malperfusion affecting late fetuses development and multi-organ infection caused by SARS-CoV-2

Behling, J.A.K.
Brain Institute of Rio Grande do Sul (BraIns), Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil

Zanirati, G.
Brain Institute of Rio Grande do Sul (BraIns), Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil

Rodrigues, F.V.F.
Brain Institute of Rio Grande do Sul (BraIns), Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil

Grahl, M.V.C.
Brain Institute of Rio Grande do Sul (BraIns), Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil

Krimberg, F.D.
Brain Institute of Rio Grande do Sul (BraIns), Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil

Pinzetta, G.
Brain Institute of Rio Grande do Sul (BraIns), Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil

Borém, L.V.B.
Graduate Program in Health Woman of Medical School of Federal University of Minas Gerais, Brazil

Savi, D.
Virchow Laboratory, Belo Horizonte, Minas Gerais, Brazil

Machado, D.C.
Brain Institute of Rio Grande do Sul (BraIns), Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil

Da Costa, J.C.
Brain Institute of Rio Grande do Sul (BraIns), Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil

Marinowic, D.R. (✉ daniel.marinowic@pucrs.br)
Brain Institute of Rio Grande do Sul (BraIns), Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil
Abstract

Background: Pregnant women are susceptible to the novel coronavirus (SARS-CoV-2) and the consequences on the fetus are still uncertain. Here, we present a case of a pregnant woman with subclinical hypothyroidism and PAI-1 4G/5G mutation who was infected with SARS-CoV-2 at the end of the third trimester of pregnancy.

Methods: nested PCR were performed to detect the virus, followed by ssDNA sequencing.

Results: transplacental transmission of SARS-CoV-2 can cause placental inflammation, ischemia and neonatal viremia, with complications such as preterm labor and damage to the placental barrier in patients with PAI-1 4G/5G mutation.

Conclusion: we show the possibility of transplacental transmission of SARS-CoV-2 infection during the last weeks of pregnancy.

Introduction

In December 2019, several cases of pneumonia emerged in the city of Wuhan, China. It was found that it was due to a new betacoronavirus SARS-CoV-2. In March 2020, the World Health Organization (WHO) declared the disease as a pandemic\(^1,^2\).

Infection with the SARS-CoV-2 virus causes Coronavirus Disease 2019 (COVID-19), whose main symptoms are fever, fatigue, and cough and may progress to dyspnea or, in more severe cases severe acute respiratory system\(^3\). Besides, older age and comorbidities, such as diabetes, respiratory disease, hypertension, severe heart disease, or immunosuppression are considered risk factors for worsening outcomes of coronavirus infection. There is evidence that pregnant women are more susceptible to respiratory pathogens including SARS and Middle East Respiratory Syndrome (MERS) which are responsible for severe complications during pregnancy\(^4,^5,^6\). Recently, few reports suggested that pregnant women are also susceptible to SARS-CoV-2\(^7,^8\). Also, a report showed that the transplacental transmission of SARS-CoV-2 is possible\(^9\).

Here we present a case of a pregnant woman with to subclinical hypothyroidism and the presence of a PAI-1 4G/5G mutation condition who was infected with SARS-CoV-2 at the end of the third trimester of pregnancy in southeast Brazil and demonstrated a vertical transmission of SARS-CoV-2. The present study discusses the details of fetal multi-organ tissue virological and pathological investigations.

Ethical Aspects

This study was approved by the Research Ethics Committee of Pontifical Catholic University of Rio Grande do Sul (PUCRS) with approval number 3.977.510. The participant provided written informed consent before inclusion in this study.
Case Report

A 36-year-old pregnant woman (first pregnancy), presented for prenatal care in a pregnancy service due to subclinical hypothyroidism and the presence of a PAI-1 4G/5G mutation. During all prenatal care there are no maternal and fetal complications. An ultrasound scan at 37 weeks of gestation to access growth and fetal well being showed good evolution. Estimated fetal weight was 2,920 g (40th centile), amniotic fluid index 14.8 cm, normal maternal and fetal Doppler, mean uterine artery PI = 0.51; Umbilical artery with positive diastolic flow and pulsatility index (PI) = 1.05; middle cerebral artery (MCA) PI = 1.19; normal cerebroplacental ratio (CPR) = 1.13. The biophysical profile is also normal (8/8).

At the 39th week of gestation, she presented spontaneous rupture of membranes before labor, meconium amniotic fluid, starting contractions of the active phase afterward. Normal cardiotocography, category 1, reassuring pattern, and clinical examination without abnormalities, normal uterine tone, satisfactory uterine dynamics, 6 cm dilated cervix, cephalic fetus. After 5 hours of adequate evolution labor with epidural analgesia, she was in the expulsive period and was necessary operative vaginal delivery (Simpson forceps) due to a non-reassuring fetal status. Birth of a newborn male, hypotonic, weighting 2,600 g. Apgar scores were 1, 1, and 4 in the 1st, 5th, and 10th minutes, respectively. Referred immediately to the neonatal intensive care unit. Evolved to death after 4 days.

The placenta and the organs were sent for autopsy analysis. Considering the extensive placental lesion that severely compromised fetal perfusion understanding that there is no evidence in the literature that the PAI 1 4G/5G mutation could justify such ischemia, taking into account the context of the current pandemic of SARS-COV-2, serology of the patient was performed 10 days after delivery with positive IgG (10.9) by the immunofluorescence method and the paraffin embedded tissue were sent to molecular investigation.

Methods

RNA extraction and reverse transcription for first-strand DNA synthesis

RNA was extracted from paraffin embedding sample of the placenta, lung, liver, heart, kidney, and brain of the fetus using a ReliaPrep™ FFPE Total RNA Miniprep System kit (PROMEGA) according to the manufacturer recommendations. The RNA control was extracted from nasopharyngeal and oropharyngeal (throat) specimens collected by a healthcare professional following the Centers for Disease Control and Prevention (CDC).

Reverse-transcriptase first-strand DNA synthesis was performed by the 3’ primer technique using M-MLV reverse transcriptase (THERMO FISHER SCIENTIFIC) with two distinct reverse primers (hCOVassay1 R: 5’AGCAGCATCACCACCATTG 3’ and hCOVassay2 R: 5’ CCGCCATTGCCAGCCAATT 3’). After the transcription reaction, the product was quantified in a NanoDrop fluorometer.
Molecular analysis using qRT-PCR

Real-time PCR was performed using StepOne Plus (THERMO FISHER SCIENTIFIC) equipment. The samples were amplified from the initial amount of over to 200 ng of ssDNA for each sample using the PowerUp SYBR Green Master Mix kit (THERMO FISHER SCIENTIFIC). The primers sequences used were hCOVassay1 – F 5'GCCTCTTCTCGTTCCTCATCAC 3' / R 5'AGCAGCATCACCGCCATTG 3' and hCOVassay2 – F 5'AGCCTCTTCTCGTTCCTCATCAC 3' / R 5'CCGCCATTGCCAGCCATTTC 3'.

Nested PCR technique

After the first reaction of RT-PCR, a new amplification was performed using the same primer set as the first PCR. For this new reaction (Nested PCR), the PCR product generated in the initial amplification was used as a template. The thermal cycles and the optimized times were the same used for the first PCR amplification.

ssDNA Sequencing

Sequencing of samples was performed by ACTGene Análises Moleculares Ltd. (Center for Biotechnology, UFRGS, Porto Alegre, RS, Brazil) using an AB 3500 Genetic Analyzer (THERMO FISHER SCIENTIFIC). Sequencing data were collected using Data Collection 3 software (THERMO FISHER SCIENTIFIC) and the resulting Data Collection files were converted into FASTA files with standard parameters. Using Clustal omega software, the FASTA files were aligned along with the complete Sars-CoV-2 genome sequences that follows: China (MT079844.1), Italy (MT890669.1), United States (MT642254.1), Russia (MT890462.1), and Brazil (MT827074.1).

Results

Autopsy and Organs Findings

The placenta and umbilical cord weighed 416.0 g and measured 42.0 x 12.0 x 4.0 cm. The placenta shape was discoid, with a firm reddish-colored maternal face, spongy in appearance, and adherent clots. It was also possible to observe diffuse whitish areas. The fetal face was smooth and opaque and showed a membrane with evident vessels.

The left lung showed intense and extensive acute bronchopneumonia, with numerous neutrophils and pyocytes filling the alveoli, along with abundant amniotic fluid, fibrin deposits, and bacterial colonies. The interlobular septa showed intense congestion and marked recent hemorrhage, forming a trabecular aspect on macroscopic examination. The right lung showed intense capillary and vascular congestion, with amniotic fluid and meconium in the alveoli. In addition to hyaline membranes covering the walls of
several alveoli and bronchioles. Several megakaryocytes were also observed in the capillary of the alveolar septa.

The heart did not demonstrate significant changes. The liver showed lobulated hepatocellular parenchyma with intense extracellular (canalicular) and more discrete intracellular cholestasis, with necrosis of the hepatocytes in the lobular center peri-vein, where recent hemorrhage and congestion were also observed. Right adrenal with 5.0 mm necrosis of the cortex, associated with recent peripheral and medullary hemorrhage. Left adrenal with intense congestion and recent hemorrhage in the cortex. Kidneys presented a mature appearance, showing intense congestion and foci of recent hemorrhage, in addition to hyaline cylinders in the tubules and diffuse acute tubular necrosis. Spleen, pancreas, and gallbladder did not show any noticeable alterations (Figure 1).

Virology investigation

It was possible to detect the presence of SARS-CoV-2 through nested RT-PCR assay in the placenta, liver, heart, lung, and brain samples. Simple RT-PCR assays did not identify the virus in any of the tissues analyzed. The amplification and melt curves are shown in figure 2. The sequencing of the generated amplicons showed a high sequence identity for different strains of SARS-CoV-2. The placenta showed of sequence identity of 100 % with query cover 46 %, the brain demonstrated an identity of 100 % with query cover 45 % and in the heart was possible observed 100 % identity with query cover 40 %. It was not possible to analyze the sequencing of the other organs due to low quantity of the material.

Discussion

Thrombophilia is a hereditary or acquired condition that can lead an individual to an increased risk of venous thromboembolism\textsuperscript{10}. Among this, there is a polymorphism in the promoter region of the PAI-1 gene. Most obstetric societies worldwide recommend avoiding plasminogen activator inhibitor-1 (PAI-1) polymorphism testing\textsuperscript{10,11}. The screening of PAI-1 should not be performed since the available studies show no evidence that there could be an influence on adverse outcomes in pregnancy or patient management, since about 50% of the general population presents this condition\textsuperscript{10}. Thus, including PAI-1 in the screening could generate anxiety and adverse effects of unnecessary conducts\textsuperscript{11}.

However, the current grand discussion is whether COVID infection can influence hereditary thrombophilias, as well as acquired thrombophilias, or even potentiate mutations that under normal conditions would not present significant changes\textsuperscript{13,14}. Although the patient's mutation, as stated earlier, is not a formal indication of prophylaxis for thromboembolism, the use of anticoagulants may be indicated according to medical criteria\textsuperscript{13,17}. The interesting thing in the report presented is that the pregnant woman used 40 mg of enoxaparin per day, until the day before she started labor signals, thus performing prophylaxis for the condition in question. It is emphasized that the analysis, both of the
placenta and organs of the newborn, had laboratory confirmation of SARS-CoV-2 infection by reliable methods, and all histopathological tests were performed by the same experienced pathologist, blind to the maternal results of SARS-CoV-2. Because it is a pregnancy that evolved to death, we found evidence of the presence of SARS-CoV-2 in several organs analyzed post mortem, as well as in the placenta, thus evidenced by transplacental transmission\textsuperscript{14,15,16}. We also do not know whether fetal viremia and the presence of SARS-CoV-2 in tissues may have influenced in some way the response to treatments performed without success\textsuperscript{13,14}. Also, other studies have shown that transplacental transmission is indeed possible in the last weeks of pregnancy\textsuperscript{12,15}. Overall, there is limited evidence of vertical transmission or significant mortality for pregnant women with COVID-19. However, several adverse perinatal outcomes have been reported, including increased risk of miscarriage, premature rupture of membranes, premature and stillbirth, and preeclampsia\textsuperscript{13,14,16,21}. In this report we highlight the non-weight gain predicted in a critical period of the 37th to 40th weeks where fetal growth is an extremely important factor for the outcome and good fetal evolution, showing complication of restricted intrauterine growth due to alterations in the cord and placental perfusion leading to hypoxia and unfavorable evolution to fetal death. This fact is corroborated by the 37-week ultrasound that presented good evolution so far with an estimated fetal weight of 2,920 g (40th percentile) the birth weight counterpart of 2,600 g at 39 weeks (near 3th percentile). This fact reinforces the hypothesis that placental injury occurred during the last weeks of pregnancy, leading to a placental flow deficiency that culminated in chronic fetal hypoxia and the expected non-weight gain\textsuperscript{21}. 

As the global epidemic continues to expand, there will be additional information available on the effects of COVID-19 on pregnant women and their newborns\textsuperscript{17}. In the unfortunate event of mortality resulting from SARS-CoV-2 infection among pregnant or newborn women, pathological evaluation of tissues together with molecular characterization of the virus would be useful in determining the pathogenesis of the disease, as is the case in many cases of emerging infections\textsuperscript{12,19}. 

In conclusion, we show the possibility of transplacental transmission of SARS-CoV-2 infection during the last weeks of pregnancy. In addition, transplacental transmission can cause placental inflammation, ischemia and neonatal viremia, with complications such as preterm labor and damage to the placental barrier in patients with the presence of PAI-1 4G / 5G mutation. Finally, further studies are needed to confirm our findings and help guide pregnancy management in women with COVID-19, especially in the last trimester of pregnancy in patients with risk or potential factors such as thrombophilias.

\textbf{Declarations} 

The authors declare no competing interests.

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