Persistence of SARS-CoV-2 in the first trimester placenta leading to transplacental transmission and fetal demise from an asymptomatic mother

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ABSTRACT: Coronavirus disease 2019 (COVID-19) is caused by infection of the respiratory tract by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which survives in the tissues during the clinical course of infection but there is limited evidence on placental infection and vertical transmission of SARS-CoV-2. The impact of COVID-19 in first trimester pregnancy remains poorly understood. Moreover, how long SARS-CoV-2 can survive in placenta is unknown. Herein, we report a case of a pregnant woman in the first trimester who tested positive for SARS-CoV-2 at 8 weeks of gestation, although her clinical course was asymptomatic. At 13 weeks of gestation, her throat swab tested negative for SARS-CoV-2 but viral RNA was detected in the placenta, and the Spike (S) proteins (S1 and S2) were immunolocalized in cytotrophoblast and syncytiotrophoblast cells of the placental villi. Histologically, the villi were generally avascular with peri-villus fibrin deposition and in some areas the syncytiotrophoblast layer appeared lysed. The decidua also had fibrin deposition with extensive leukocyte infiltration suggestive of inflammation. The SARS-CoV-2 crossed the placental barrier, as the viral RNA was detected in the amniotic fluid and the S proteins were detected in the fetal membrane. Ultrasonography revealed extensively subcutaneous edema with pleural effusion suggestive of hydrops fetalis and the absence of cardiac activity indicated fetal demise. This is the first study to provide concrete evidence of persistent placental infection of SARS-CoV-2 and its congenital transmission is associated with hydrops fetalis and intrauterine fetal demise in early pregnancy.

Key words: virus / COVID-19 / coronavirus / pregnancy / vertical transmission / abortion / hydrops fetalis / congenital / placenta / fetal membrane

Introduction

In March 2020, the World Health Organization declared coronavirus disease 2019 (COVID-19) as a global pandemic. Caused by infection of the respiratory tract with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the numbers of confirmed cases have escalated rapidly. Despite this, the information on the effects of SARS-CoV-2 infection in pregnancy is limited to description of
presenting symptoms and outcomes during late trimester infections (Allotey et al., 2020; Gajbhiye et al., 2020; Waghmare et al., 2021). Since the maternal physiology changes dynamically throughout pregnancy, the information on late trimester infections cannot be extrapolated directly to infection earlier in gestation.

During pregnancy, the placenta is an anatomical barrier that acts as the first line of defense to avoid transmission of pathogens from mother to fetus. However, some infectious agents can breach the placental barrier resulting in vertical transmission and activating innate immune responses causing major maternal and fetal complications (Parker et al., 2020). For pregnant women with COVID-19, vertical transmission of SARS-CoV-2 and the pregnancy complications like miscarriage, fetal malformations and/or stillbirth, are potentially serious concerns. The spike (S) proteins of SARS-CoV-2 bind to the host receptor ACE2 allowing the virus to enter the cells and replicate (Hoffmann et al., 2020; Jagtap et al., 2020). The placental syncytiotrophoblast, cytotrophoblasts and the extra-villous trophoblasts express the ACE2 receptors and also the genes necessary for S protein processing, viral replication and budding (Ashary et al., 2020; Colaco et al., 2020; Singh et al., 2020; Taglauer et al., 2020). These observations imply that the placenta is permissive for SARS-CoV-2 infection. Indeed, SARS-CoV-2 RNA and protein are detected in placenta of mothers with COVID-19 (Ashary et al., 2020; Hosier et al., 2020; Taglauer et al., 2020; Vivanti et al., 2020).

In clinical cases and experimentally induced infections, SARS-CoV-2 is detected in multiple tissues (Best Rocha et al., 2020; Bradley et al., 2020; Chandrashekar et al., 2020; Costa et al., 2020; Yao et al., 2020; Zhang et al., 2020) during acute viremia; extended periods of viral shedding after clearing pulmonary infection have also been observed (Gupta et al., 2020; Wu et al., 2020; Zhang et al., 2020). Whether SARS-CoV-2 can also survive and shed from the placenta for extended periods is hitherto unknown.

Congenital infection of SARS-CoV-2 from feto-maternal interface is a matter of controversy. Some case series suggest that there is lack of vertical transmission of SARS-CoV-2 (Masmejan et al., 2020; Li et al., 2020b). However, large-scale systematic reviews reveal that a proportion of babies born to mothers with COVID-19 are positive for viral RNA (Allotey et al., 2020; Gajbhiye et al., 2020). Whether this infection is due to transmission of SARS-CoV-2 in utero or the infection is acquired during the course of delivery or after birth remains unclear.

Herein, we report a case of first trimester asymptomatic SARS-CoV-2 infection associated with hydrops fetalis and fetal death. We observed that the viremia persisted in the placenta weeks after the mother cleared it from the pulmonary tract and there was transplacental transmission as SARS-CoV-2 could be detected in the amniotic fluid and fetal membranes.

**Materials and methods**

**Ethics statement**

The study was approved by the Institutional Ethics Committee of ESI-PGIMSR and Model Hospital, Mumbai, India. Written informed consent was taken from the patient.

This case is described in the preprint (Shende et al., 2020). A woman with 13 weeks of amenorrhea reported for routine antenatal checkup at the hospital. She had history of asymptomatic SARS-CoV-2 infection in the nasopharyngeal swabs 5 weeks ago. Upon examination, ultrasound evaluation showed hydrops fetalis with fetal demise. Dilatation and curettage were done according to hospital norms and necessary precautions were taken to avoid contamination of the placental and amniotic fluid. Clear amniotic fluid was carefully aspirated from the gestational sac and transported in a sterile container. Serum was sent for TORCH (Toxoplasmosis, Rubella Cytomegalovirus, Herpes Simplex), antiphospholipid (APLA) IgG and IgM, anti-double strand DNA, cardiolipin (ACL) IgG and IgM testing. Pieces of the placental tissue were fixed in 10% buffer formalin processed for paraffin embedding and sectioning. Placental histopathology was evaluated by routine hematoxylin and eosin staining. Fluorescence in situ hybridization on paraffin sections was done to determine aneuploidy of chromosomes 13, 18, 21, X and Y as described previously (Modi et al., 2003). Placental villi were collected in virus transport medium. Total RNA was isolated from placental villi using the Trizol reagent as per manufacturer’s instructions (Thermo Fisher Scientific, Waltham, MA, USA). To test the presence of SARS-CoV-2, reverse transcriptase polymerase chain reaction (RT-PCR) for ORF1b, RdRP, N and E genes was done using commercial kits. Immunofluorescence was done as described earlier with minor modifications (Godbole et al., 2017). The SARS-CoV-2 spike proteins S1 and S2 were detected using 1:25 dilution of the monoclonal antibodies (MP Biomedicals, Catalogue number: SKU: 08720301, SKU: 08720411, Asia Pacific, Singapore) and the Opal 7 kit (catalog #NEL797001KT; PerkinElmer, Waltham, MA, USA), which uses individual tyramide signal amplification-conjugated fluorophores was used for signal detection.

**Results**

**Case details**

A 26-year-old woman, third gravida, one living child and one first trimester spontaneous abortion reported to the hospital after a positive pregnancy test. She had conceived spontaneously with no history of any medical or surgical comorbidities. Her obstetric ultrasonography report revealed a gestational age of 7.6 weeks. Three days later she came in contact with a symptomatic COVID-19 positive patient and hence was immediately tested for SARS-CoV-2 in the nasopharyngeal swab by RT-PCR. The throat swab was positive for ORF1b gene of SARS-CoV-2 with a Ct value of 28 (Table I). She was asymptomatic and hence was quarantined for 10 days at a local hospital. She was discharged with further advice of home quarantine for next 7 days. She remained asymptomatic throughout this period.

Five weeks after the first ultrasound (expected 13 weeks of gestation), she reported for antenatal care where she was advised routine blood investigations along with ultrasonography for nuchal translucency. The ultrasound evaluation showed hydrops fetalis along with fetal demise. Since there was fetal demise, the products of conception were sent for further routine evaluation and SARS-CoV-2 testing. The repeat throat swab taken on the day prior of dilation and curettage was negative for SARS-CoV-2 (Table I).
Table I  RT-PCR for SARS-CoV-2 in the nasopharyngeal swabs, placenta and amniotic fluid from a pregnant woman with asymptomatic COVID-19 in first trimester.

| Tissue                                      | Gene tested | Ct value | Result   | Load          |
|---------------------------------------------|-------------|----------|----------|---------------|
| Maternal nasopharyngeal swab (I)            | ORF1b       | 28       | Positive | Moderate      |
| Maternal nasopharyngeal swab (II)           | ORF1b       | ND       | Negative | Absent        |
| Placental tissue transport medium           | E gene      | 28.4     | Positive | Moderate      |
|                                            | RdRp        | 27.5     |          |               |
| Placenta                                   | E gene      | 34.0     | Positive | Mild to moderate |
|                                            | N gene      | 38.26    |          |               |
| Amniotic fluid                              | E gene      | 26.3     | Positive | Moderate      |
|                                            | RdRp        | 23.4     |          |               |
| Negative control                            | E gene      | ND       | Negative | Absent        |
|                                            | N gene      | ND       |          |               |
|                                            | ORF1b       | ND       |          |               |
|                                            | RdRp        | ND       |          |               |

Maternal nasopharyngeal swab (I) is maternal swab collected at 8 weeks of gestation during active infection. Maternal nasopharyngeal swab (II) is nasopharyngeal swab collected at 13 weeks of gestation at the time of abortion. Placental tissue transport medium is the viral transport medium in which the placental villi were incubated overnight. Negative control is from the kit. Loads are interpreted as per kit standards. ND is below detectable range. Ct values are cycle threshold value for each sample.

First trimester SARS-CoV-2 infection is associated with hydrops fetalis and fetal demise

At 8 weeks of amenorrhea, ultrasonography revealed a single live fetus with a crown rump length (CRL) of 14.6 mm corresponding to 7.6 weeks of gestation. Five weeks later, the second ultrasound evaluation revealed a CRL of 38 mm corresponding to 10.5 weeks of gestation (Fig. 1). There was extensive bilateral pleural effusion and subcutaneous edema suggestive of hydrops fetalis (Fig. 1). There was no cardiac activity or blood flow in the fetal heart suggestive of fetal demise (Fig. 1).

Serum antiphospholipid, anti-nuclear and anticardiolipin, TORCH screening and chromosomal aneuploidies in placenta

Her blood group was O+ve ruling out possibility of autoimmune cause of hydrops. Fluorescence in situ hybridization of placental villi showed disomy for 13, 18, 21, and X chromosomes ruling out possibility of major chromosome aneuploidies causing hydrops fetalis (not shown). Serum testing revealed that she was negative for antiphospholipid, anti-nuclear and anticardiolipin antibodies. She was also negative for IgM antibodies of TORCH which indicated absence of active infection. However, she was positive for IgG antibodies against Toxoplasma gondii, Rubella, Cytomegalovirus and Herpes simplex virus I (Supplementary Table S1) suggestive of past infection.

Presence of SARS-CoV-2 in placental cells

RNA for both E gene and RdRp gene of SARS-CoV-2 were detected in the supernatants of placental villi incubated in virus transport medium. The Ct value for E gene was 28.4 and RdRp gene was 27.5, suggestive of moderate viral load (Table I). In the total RNA from placental villi, the E and the N genes of SARS-CoV-2 were detected at

Figure 1. Transabdominal ultrasound at 13 weeks of amenorrhea showing hydrops fetalis and demise in a pregnant woman who was positive for SARS-CoV-2 at 8 weeks of gestation. (A) Extensive subcutaneous edema (white arrows) and pleural effusion (PE). (B) Images showing absent cardiac activity. (C) Absence of blood flow in the fetal heart (arrow).
Ct values of 34.0 and 38.2, respectively suggestive of low to moderate viral carriage (Table I). To localize SARS-CoV-2 in placental cells we carried out immunofluorescence using monoclonal antibodies against the spike protein S1 and S2 on placental sections (Fig. 2). Both, S1 and S2 proteins were diffusely localized mainly in the cytoplasm of syncytiotrophoblast and some cytotrophoblast cells. Some villus stromal cells were also positive for both the proteins. In some syncytiotrophoblast cells, S2 was also found as aggregates (Fig. 2).

**Histopathology of the placenta**

Placental histopathology (Fig. 3) revealed conspicuously avascular villi with extensive perivascular fibrin deposition. The intravillous area had large numbers of stromal cells and extensive vacuolation. In some areas, the syncytiotrophoblast layer appeared lysed. The decidua also had extensive fibrin deposition and large dilated blood vessels engorged with blood. There were signs of widespread inflammation as evident by presence of large numbers of leukocytes including the polymorphonuclear leukocytes in the decidual bed as well as in the intervillous spaces. A comparative assessment of the histological features of first trimester feto-maternal interface and that observed in the present case is shown in Supplementary Table SII).

**Presence of SARS-CoV-2 virus in amniotic fluid and fetal membranes**

To test if SARS-CoV-2 crosses the placental barrier, we carried out RT-PCR for viral RNA in the amniotic fluid and immunofluorescence of spike proteins in fetal membrane. In the amniotic fluid aspirate, the Ct value for the E gene was 26.3 and the RdRp gene 25.4 (Table I). By immunofluorescence, the viral S proteins were also detected in cells of fetal membrane where both S1 and S2 were diffusely localized in cytoplasm (Fig. 4).

**Discussion**

To our knowledge, this is the first case demonstrating persistence of SARS-CoV-2 in the placenta from an asymptomatic mother in the first trimester. This was associated with hydrops fetalis and fetal demise. The study also provides evidence for transmission of the virus from the placenta to the amniotic fluid and fetal membrane. Prior studies have shown the presence of viral RNA in tissues during the clinical course of active COVID-19 infection. Herein, we show that while the mother was negative for SARS-CoV-2 in the pulmonary passage, extensive viremia was observed in the placenta. SARS-CoV-2

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**Figure 2.** Localization of spike proteins of SARS-CoV-2 in villi of first trimester placenta of a women with asymptomatic COVID-19 at 8 weeks of gestation. Paraffin sections were immunostained for spike proteins S1 (A) and S2 (B) of SARS-CoV-2 using monoclonal antibodies. Boxed area is enlarged in the next panel to show the specific cell types. Negative are sections of same tissues incubated without primary antibody. In all the sections the red staining in the inter villus spaces is autofluorescence of red blood cells. Scale bar represents 100 μm.
RNA was detected in the supernatant of viral transport medium containing the placental villi and also in total RNA extracted from placentas. Along with viral RNA, the viral proteins could be readily detected in the cytotrophoblasts, syncytiotrophoblast and some villus stromal cells. Although, a lag in systemic viremia and placental infection is known, in the present study, the woman was detected positive for SARS-CoV-2 in the respiratory tract at 8 weeks of gestation, the placentas was positive 5 weeks later. At this time, her throat swab was negative for the virus. These results imply that the virus persisted in the placental tissue weeks after it was cleared from the pulmonary passage.

Earlier studies have shown that intracellular detection of SARS-CoV spike proteins is indicative of viral replication and budding within the cells (Stertz et al., 2007). Herein, we observed that the SARS-CoV-2 spike proteins are detected in the cytoplasm of the cytotrophoblasts, syncytiotrophoblast and some stromal cells of the first trimester villi weeks after the pulmonary infection was cleared. In women with active COVID-19 in the third trimester, the spike proteins are localized in cytoplasm of the placental cells (Taglauer et al., 2020). These results together tempt us to suggest that the virus not only infects but also replicates in the placenta. Indeed, by analyzing single-cell RNAseq data, we have shown that the ACE2 positive placental cells express mRNA for proteins involved in viral budding and replication (Ashary et al., 2020). While it will be of interest to detect subgenomic RNA to study viral replication dynamics in the placental cells, we did detect the presence of E and N gene of SARS-CoV-2 in the RNA extracted from the placenta suggestive of viral replication. Thus, we propose that the placenta is a permissive site for SARS-CoV-2 replication and to the best of our knowledge this is the first study demonstrating such long-term presence of the virus in any tissues post clearance from the pulmonary sites.

The viremia of the placental cells was further coupled with extensive inflammation as deduced by leukocyte infiltration in the intravillous spaces and decidua, fibrin deposition and lysis of syncytiotrophoblast of the villus cells. Inflammatory changes and fetal vascular malperfusion or fetal vascular thrombosis are reported in second and term placental tissues obtained from mothers infected with COVID-19 and exhibiting active viremia (Baergen and Heller, 2020; Hosier et al., 2020; Shanes et al., 2020; Vivanti et al., 2020). These observations imply that SARS-CoV-2 does not just reside in placental cells but also elicits an inflammatory response. This is not surprising as ACE2 positive cells in the placenta abundantly expressed genes in the Toll-like receptor pathway and were also enriched for components of the other pathways that drive inflammation (Ashary et al., 2020).

How the pulmonary virus reaches extrapulmonary sites such as the placenta is an enigma. Although coronaviruses usually infect the respiratory tract, viral shedding in blood circulation is known and circulating lymphocytes are also known to be infected by SARS-CoV-2
fetalis is most often a complication of Rh incompatibility where the mother is Rh negative and an immune reaction could trigger fetal hydrops. However, in this case, the mother was Rh positive ruling out immune hydrops fetalis. A number of chromosome abnormalities are associated with nonimmune hydrops fetalis (Norton et al., 2015; Kosinski et al., 2020). In the present case, the placenta was disomic for chromosomes 13, 18, 21, and X ruling out these factors in etiology of hydrops; although other rare genetic causes of hydrops are not ruled out. Vertical transmission of many types of virus is known to cause fetal hydrops; however, no such association has been reported for coronavirus. In our case the mother was negative for IgM of herpes simplex virus and Toxoplasma gondii, ruling out active infection with these organisms. She was also negative for antiphospholipid, antinuclear and anticardiolipin antibodies which are associated with spontaneous abortions. Thus, it appears that SARS-CoV-2 infection is the most likely cause of fetal hydrops and fetal demise in the present case.

**Conclusion**

This is the first study to provide evidence of persistent tissue infection of SARS-CoV-2 and its congenital transplacental transmission in early pregnancy associated with hydrops fetalis and fetal demise. Further studies are required to throw more light on vertical transmission of SARS-CoV-2 infection in first trimester so that universal screening of all pregnant women can be considered in such cases to avoid adverse fetal outcome.

**Data availability**

The associated data is made available in the manuscript.

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**Authors’ roles**

P.S., P.G. and P.U. were involved in, case identification, diagnosis, clinical workup and patient management. M.G. did the clinical laboratory investigations and interpreted the data. V.P., S.B. and V.B. were involved in SARS-CoV-2 RT-PCR. A.B. and D.M. performed the histopathology and immunofluorescence and interpreted the data. R.G. and S.M. coordinate the work and helped in data analysis. P.S., R.G., D.M. and A.B. wrote the first draft. D.M., S.M., R.G., A.B. and P.G. edited the drafts. All the authors approved the final version of the manuscript.

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