DOI: 10.1111/pin.13214

LETTER TO THE EDITOR

Pathology International WILEY

C4d deposition and CD39 downregulation in the placental infection by SARS-CoV-2

To the Editor,

The majority of placental pathology studies did not observe evidence of infection in the placenta of women with COVID-19 who delivered neonates. In contrast, placentas of transmitted cases showed chronic histiocytic intervillositis (CHI) with syncytiotrophoblast necrosis.[1] Recently, standard definition of placental infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is proposed as the identification of viral antigens using immunohistochemistry or RNA in situ hybridization.[2] Complement deposition (C4d) deposition in the placenta has been reported in systemic lupus erythematosus, CHI and placental infection by SARS-CoV-2 infection.[3] A healthy placenta expresses CD39, an ectonucleotidase that protects against inflammatory stress and thrombus formation. We previously demonstrated CD39 downregulation on the surface of villi in the CHI.[4] In this report, we describe a case of pregnancy complicated by SARS-CoV-2 infection and the accompanying placental findings with C4d deposition and CD39 expression.

A 23-year-old woman (gravida 2, para 1) was referred to another hospital because of fever at 30 weeks and 4 days of gestation. She tested positive on the nasopharyngeal swab RT-PCR test for SARS-CoV-2. The patient was transferred to our hospital because of increased uterine contraction at 32 weeks and 0 days of gestation. Her body temperature was 36.4°C, and she had no respiratory symptoms. Elevated D-dimer (27.0 μg/mL) and C-reactive protein (3.67 mg/dL) were observed upon admission to our hospital. The fetal heart rate (FHR) pattern showed no baseline FHR variability or late decelerations. On the same day of admission, a female neonate was born via emergency cesarean delivery due to non-reassuring fetal status. The neonate weighed 1495 g. Apgar scores were 1, 5, and 8 at 1, 5, and 10 min after birth, respectively. Cord arterial blood gas was acidic (pH 6.915). The infant was immediately separated from her mother and transferred to the neonatal intensive care unit. The neonate tested negative on the nasopharyngeal swab RT-PCR test for SARS-CoV-2 at 0, 48 h, and 15 days after birth. Ultrasound examination revealed intraventricular hemorrhage, but neurological abnormalities were not detected. The infant was discharged in good clinical condition at 49 days after birth. Our institution does not require approval from an Ethics Committee.

The placenta was 18 × 15 × 2 cm and weighed 280 g. The cut surface showed irregular strands of pale yellow-white indurations (Figure 1a). Histological examination revealed infiltration of many chronic inflammatory cells and neutrophils in the intervillous space (Figure 1b). Perivillous fibrin deposition and necrosis of syncytiotrophoblasts were also observed. No apparent infarction or thrombus formation was observed. The umbilical cord, decidua, and membrane were unremarkable. Immunohistochemical staining of the control and the placentas in this case were performed using CD68, CD3, CD15, SARS-CoV-2 spike protein, C4d, and CD39. The control placenta was from the mother with SARS-CoV-2 infection, and this placenta did not show intervillositis. The number of CD68-, CD3-or CD15-positive cells in the intervillous space was evaluated. Three counts were performed at 400 magnification (high-power field), and the mean was calculated. In this study, the CD39-positive or C4d-positive villi signal was quantified as the percentage of the total number of villi in three high-power fields (400× magnification). In the control placenta, few CD68-, CD3- or CD15-positive cells were noted in the intervillous space (data not shown). In contrast, many CD68-, CD3-, and CD15-positive cells were observed in the intervillous space in the present case (Figure 1c). The percentages of CD68-, CD3-, and CD15-positive cells were 47%, 23%, and 30%, respectively in the present case. Granular and patchy staining with anti-spike protein antibody for SARS-CoV-2 were observed in syncytiotrophoblasts in the present case (Figure 1d), but not in the control. Few inflammatory cells positive for spike protein were noted in the intervillous space and fetal vessels in the present case. On the other hand, C4d deposits were not observed in the control placenta, and almost all villi showed CD39 strong and diffuse expression (data not shown). Diffuse and strong C4d deposits were present at the surface in the present case (95%, Figure 1e). Contrarily, CD39 expression was decreased at the surface of the terminal villi in the present cases (13%, Figure 1f).

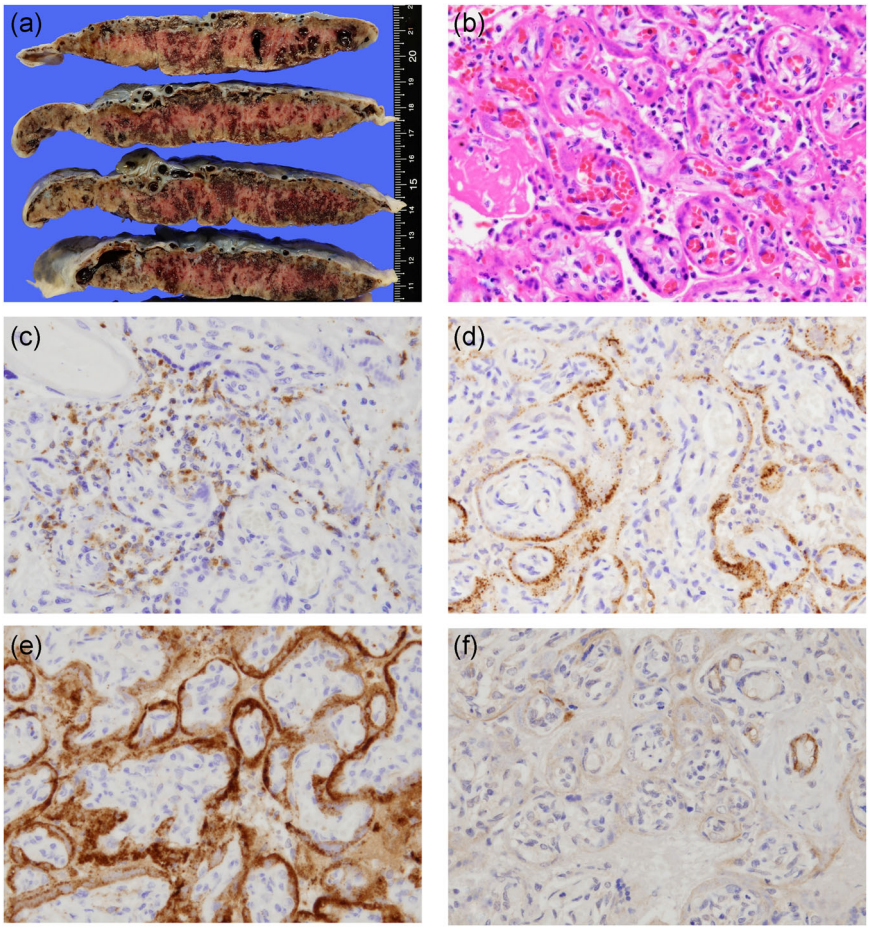

FIGURE 1 (a) Cut surface of the placenta, (b) histological findings of the placental tissue, (c) anti-CD68 immunostaining, (d) anti-spike protein for severe acute respiratory syndrome coronavirus 2, (e) andi-complement deposition immunostaining, (f) anti-CD39 immunostaining

In this report, we found diffused and strong C4d depositions at the surface of the chorionic villi of the placenta in SARS-CoV-2 vertical transmission. C4d is commonly referred to as the "footprint" of activation of the compliment system tissue injury. Moreover, the placenta showed many lymphocytes in the intervillous space with syncytiotrophoblast necrosis, which is similar to CHI. Recently Watkins et al. reposted that five of six placentas with SARS-CoV-2 infection demonstrated C4d expression at the syncytiotrophoblast surface.[3] Taken together these findings, activation of the compliment system may induce placental tissue injury with SARS-CoV-2 vertical transmission. Several studies have reported strong and diffuse expression of CD39 in the syncytiotrophoblasts of the terminal villi in healthy pregnancies.[4] In the present case, the placenta with SARS-CoV-2 infection showed a significant decrease in CD39 expression in the syncytiotrophoblasts. In addition, fibrin deposition was observed on the villi surface. CD39 is an ectonuleotidase that inhibits thrombus formation in the normal placenta. These findings suggest that intervillositis affects CD39 downregulation and intervillous fibrin deposition in the placenta during SARS-CoV-2 infection. Although the etiology of many CHI cases is unknown, some reports have demonstrated an association between CHI and infectious conditions. Debelenko et al.[5] compared the histological findings of 75 placentas from SARS-CoV-2 non-infected and infected women. Although there was no significant difference between the frequency of CHI of the two groups (4% vs. 3%, $p = 0.655$), many cases of the placentas with SARS-CoV-2 infection showed CHI.[1–3] We think that CHI is an important histological finding in the placenta of SARS-CoV-2 infected women, and immunohistochemical or in situ hybridization (ISH) study should be necessary examination for detection of viral antigen.

Although maternal symptoms of COVID-19 are generally mild, but several studies demonstrated fetal sudden death.[1–3] Previous reported cases also showed abnormal FHR, decreased fetal movement, fever and hypotonia, and another had respiratory distress. The present case showed abnormal fetal heart sound and ventricular hemorrhage. A vertically transmitted placenta may still affect the fetal condition, and some cases may lead to stillbirth. We think that it is important to emphasize the need for caution in the maternal management of SARS-CoV-2 infection regarding the possibility of sudden change.

ACKNOWLEDGMENTS

We thank Takako Tokumitsu and Nahoko Udatsu for their excellent technical assistance. This study was supported by the Japan Society for the Promotion of Science (JP21K07775). We would also like to thank Editage (www.editage.jp) for the English language editing.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Yoshiya Shimao and Yuichiro Sato designed the project. Aya Yamauchi, Teruo Ohtsuka, Yuki Kodama, and Naoshi Yamada collected clinical data. Yoshiya Shimao and Yuichiro Sato analyzed the histological and reference data. Yuichiro Sato, Yoshiya Shimao, and Yujiro Asada wrote, edited, and reviewed the manuscript.

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