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Clinical and serological findings in pregnant women and newborns: patterns of coronavirus disease 2019 placental histopathology

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SUMMARY

OBJECTIVE: The objective of this study was to evaluate the correlation between clinical and serological findings of pregnant women and newborns with patterns of histopathologic changes of the placenta diagnosed with coronavirus disease 2019.

METHODS: A prospective descriptive study was conducted with pregnant women who were positive for SARS-CoV-2 by reverse transcription polymerase chain reaction or serology (IgG and IgM). Clinical analyses were performed using ELISA to detect anti-SARS-CoV-2 IgG and IgA antibodies using the S1 spike protein domain with the Euroimmun kit. Histopathologic analyses of placentas were performed by two expert pathologists.

RESULTS: Maternal SARS-CoV-2 infection was associated with increased neonatal hospital length of stay (p=0.03), increased preterm birth (p=0.04), and Apgar score<7 at 1st min (p=0.00) and 5th min (p=0.02). Pregnant women with positive IgG and/or IgA at delivery had a higher incidence of placental histopathologic changes in addition to a greater likelihood of having an IgG-positive fetus (p<0.0001). Placentas with positive reverse transcription polymerase chain reaction for SARS-CoV-2 had a higher incidence of histopathologic changes such as maternal vascular hypoperfusion changes (p=0.00).

CONCLUSION: Maternal SARS-CoV-2 infection was associated with adverse perinatal outcomes. Pregnant women with positive IgG at delivery had a higher incidence of placental histopathologic changes. Placentas with positive reverse transcription polymerase chain reaction for SARS-CoV-2 had a higher incidence of histopathologic changes such as maternal vascular hypoperfusion.

KEYWORDS: COVID-19. Placenta. Vertical infectious disease transmission. Pathology. Serology.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is caused by SARS-CoV-2, a virus that can cause moderate to severe infections in humans. Since the virus emerged in China, in December 2019, the disease has quickly spread around the world, being declared as a pandemic by the World Health Organization in January 2020[1]. Shortly after the emergence of COVID-19, there have been reports of infections occurring in pregnant women[2], with the majority of patients being asymptomatic or with a mild disease[3].

Physiological, immunological, and mechanical changes during pregnancy increase susceptibility to infections in general, especially when the cardiorespiratory system is affected, and there is a rapid progression to respiratory failure[4,5]. Since this, epidemiological evidence indicates that pregnant women are at greater risk of developing severe disease and mortality from viral infections such as influenza, Ebola, and Lassa fever[6].

Newborns can be indirectly affected by SARS-CoV-2, through the impact of maternal COVID-19 during pregnancy, thus leading to premature childbirth. Vertical transmission is considered rare, and postnatal infections are similarly observed in breastfed and formula-fed infants. Despite the intense research, it is still unclear why neonates primarily present mild symptoms and have lower mortality rates[7].

Studies have already been carried out to assess the susceptibility of the placenta to SARS-CoV-2 infection[8,9]. This virus uses the cell receptor angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease enzyme 2 (TMPRSS2) to enter the cell host. In pregnant women, these receptors are poorly expressed in the placenta, which seemed to decrease the

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chance of vertical transmission of the virus[9], but other studies showed that placental infection is possible[10,11]. Schwartz et al.[12] analyzed 68 placentas of women diagnosed with COVID-19 during pregnancy. It was observed that virus-infected placentas had a significantly different pattern of pathological findings from uninfected placentas, regardless of the state of newborn infection. Placentitis by SARS-CoV-2, defined by the coexistence of three microscopic findings, i.e., "COVID triad"—intervillositis chronic histiocytic disease, increased fibrin, and trophoblast necrosis—were associated with stillbirths and/or neonatal deaths.

The objective of this study was to evaluate the correlation between clinical and serological findings in pregnant women and newborns with placental histopathologic patterns diagnosed with COVID-19.

METHODS

The study was carried out at the Clínica Perinatal, Rio de Janeiro, RJ, Brazil. A prospective descriptive study was carried out through the histopathologic evaluation of pregnant women's placentas diagnosed with COVID-19 in the period from April 2020 to August 2021.

Patients were recruited in the study at two different periods: (1) during pregnancy, after the confirmed diagnosis of COVID-19 and (2) at the moment of delivery, including pregnant women who were positive and verified the result for SARS-CoV-2 in any trimester, through reverse transcription polymerase chain reaction (RT-PCR) or serology (IgG and IgM) in those who did not perform RT-PCR. At the time of delivery, the placenta and samples of maternal and umbilical cord blood were collected to perform IgG and IgA serologies.

Clinical analyses were performed using the ELISA method to detect anti-SARS-CoV-2 IgG and IgA antibodies using the S1 spike protein domain with the Euroimmun kit. Histopathologic analyses of placentas were performed jointly by two expert pathologists in fetal-placental pathology with reference to the criteria of the Consensus of Amsterdam[13].

Maternal data demographics included age, race, gestational age at delivery, comorbidities, and gestational trimester in which SARS-CoV-2 infection occurred. Newborns' outcomes evaluated were small for gestational age (SGA), prematurity, Apgar score<7 at 1st and 5th min, perinatal death, and length of stay of the neonate in intensive care unit (ICU). The evaluation of outcomes included the period from delivery to hospital discharge of the newborn.

To analyze the association between numerical and categorical variables, the Mann-Whitney test was used. Chi-square and Fisher's exact tests were used to analyze the associations between categorical variables. Analyses were performed using the free program R version 3.6.1, accepting the results with a value of $p<0.05$ as significant.

This study is part of another study called Research Network in SARS-CoV-2/COVID-19 in Clínica Perinatal, in progress at IFF/Fiocruz, CAAE 30598020.0.0000.5269, and at the D'Or Institute for Research and Education, CAAE 34268020.53003.5249. In all cases, the patients signed the consent form.

RESULTS

Profile of the studied group

For this study, 152 patients were recruited, of whom 6 were not selected (3 twin pregnancies and 3 fetuses presenting malformations), totaling 146 pregnant women included in the study. No patient was excluded.

Considering the total of 146 patients evaluated in the study, it was observed that 66% (97 patients) were white and the mean age was 34.2 years (ranging from 19 to 44 years). Notably, 75 patients (51.3%) had comorbidities, the most frequently observed being diabetes (type 1, type 2, or gestational) and arterial hypertension (chronic hypertension, preeclampsia, and gestational hypertension). Seven patients (4.7%) were vaccinated against COVID-19 during pregnancy, four were immunized with Pfizer, and three with AstraZeneca.

At delivery, the mean gestational age was 38.4 weeks. Infection by COVID-19 was observed with higher prevalence in the third trimester of pregnancy (54%), and 25% tested positive at delivery, either by RT-PCR or by serology. Cesarean section was performed in 84% of cases, and delivery was preterm in 14.3%.

Placental infection and histopathology

Of the total of patients with positive diagnoses for COVID-19 during pregnancy, only 12 placentas had viral particles detected by the RT-PCR on fresh material. Results showed that there was no significant difference ($p=0.10$) between the gestational age at which the patient contracted the COVID-19 virus and the test result.

The evaluation of the moment when the pregnant woman contracted the disease during pregnancy is not correlated with the finding of the "COVID triad" ($p=0.90$), maternal vascular hypoperfusion ($p=0.67$), inflammation ($p=0.33$), acute fetal vascular hypoperfusion ($p=0.71$), and chronic fetal vascular hypoperfusion ($p=0.62$).

Placentas that presented positive RT-PCR test results for COVID-19 were evaluated to verify if they presented more histopathologic alterations than the placentas negative for

the presence of viral particles. It was observed that the positive RT-PCR test results did not increase the observation of the "COVID triad" (p=0.13), inflammation (p=0.12), acute fetal vascular hypoperfusion (p=0.73), and chronic fetal vascular hypoperfusion (p=0.42), but an association was observed between positive RT-PCR test results and maternal vascular hypoperfusion (p=0.00) (Figure 1).

Perinatal outcomes

It was observed that the presence of maternal comorbidities increased the risk of newborns requiring ICU after delivery (p=0.00).

Of the total of 140 newborns, only 3 were considered SGA, not configuring the relationship between these two variables. Chronic fetal vascular hypoperfusion was associated with increased hospitalization time of newborns (p=0.03), increase in preterm births (p=0.04), as well as Apgar score<7 in 1st (p=0.00) and 5th min (p=0.02).

In this study, two cases of fetal death of pregnant women were observed in the duration of SARS-CoV-2 infection. The first case was 38-year-old, with 38 weeks and 6 days of gestation, and diagnosis of COVID-19 was carried out at the time of delivery through RT-PCR. The placenta showed the presence of viral particles detected by the RT-PCR method in fresh material, with positive immunohistochemistry (Figure 2) and acute fetal vascular hypoperfusion, maternal vascular hypoperfusion, and inflammation. The second case occurred in 41-year-old, with 36 weeks and 3 days of gestation, previously vaccinated with AstraZeneca. COVID-19 diagnosis was performed at the time of delivery through positive results for IgG and IgA and the placenta showed alterations of maternal vascular hypoperfusion and inflammation.

Maternal and fetal serology

Maternal vaccination against COVID-19 did not change the serology of the newborn, for both IgG (p=0.25) and IgA (p=0.0), and this fact may be related to the small number of patients who were immunized during the study.

The serological evaluation of the patients and their newborns showed that when the mother presented reactive serology for IgG, there was a greater risk (p<0.0001) that the fetus was also reactive for the same immunoglobulin. A similar result was observed when evaluating the serology of newborns of IgA-reactive mothers. On the contrary, maternal seropositivity for IgG did not increase the risk (p=1.0) of the fetus being reactive for IgA, i.e., it did not increase the risk of the child being born infected with the disease. Similarly, patients with reactive IgA did not increase the risk (p=1.0) of the fetus being born infected with COVID-19, with reactive IgA.

The evaluation of maternal serologies (IgG and IgA) showed that the reagent result for IgG increased the observation of the "COVID triad" in the placentas (p=0.02) and the IgA reagent result increased the observation of acute fetal vascular hypoperfusion (Figure 3). However, both IgG-reactive serology and IgA did not show a greater risk of observing maternal vascular hypoperfusion (p=0.64 and p=0.39 for IgG and IgA,

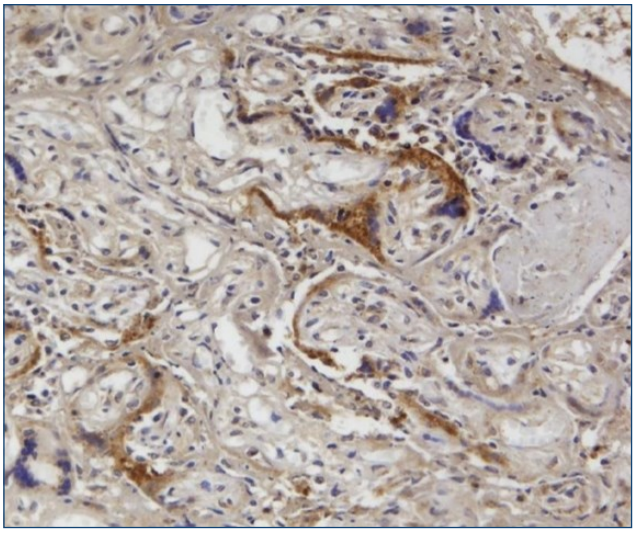

Figure 2. Immunohistochemical staining for SARS-CoV-2 viral particle. Positivity (dark brown) in the cytoplasm of trophoblastic cells: 100× magnification.

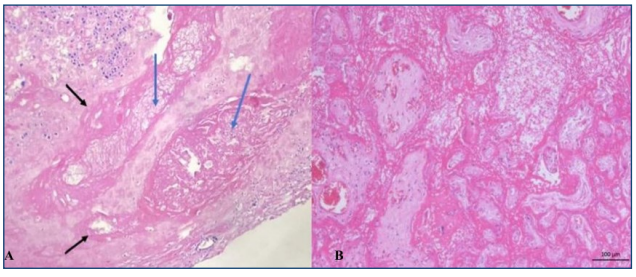

Figure 1. Changes in maternal vascular hypoperfusion. (A) Thrombosis (blue arrow) and fibrinoid necrosis (black arrow) of the decidual vessel. (B) Massive deposition of perivillous fibrin with trophoblast necrosis=area of infarction (hematoxylin-eosin, original 100× magnification).

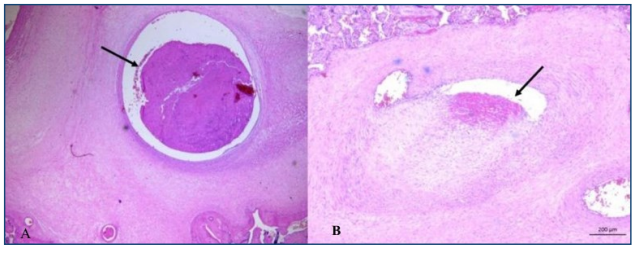

Figure 3. Acute fetal vascular hypoperfusion changes. (A) Occlusive thrombosis of chorionic plaque vessel (hematoxylin-eosin, original 100× magnification). (B) Subintimal fibrinoid degeneration of the villous trunk vessel wall (hematoxylin-eosin, original 100× magnification).

respectively), inflammatory (p=0.20 and p=0.40 for IgG and IgA, respectively), and chronic fetal vascular hypoperfusion (p=0.60 and p=0.14 for IgG and IgA, respectively).

Pregnant women's placentas positive for COVID-19 were also evaluated. It was observed that patients with the "COVID triad" did not have IgG-reactive newborns (p=0.54) or IgA (p=1.0). However, patients with changes in maternal vascular hypoperfusion had newborns with reactive serology for IgG (p=0.04), and patients with alterations in acute fetal vascular hypoperfusion had newborns with reactive serology for IgA (p=0.02).

DISCUSSION

Relationship between neonatal outcomes and histopathologic findings

Studies have described histopathologic evidence of poor fetal and maternal vascular perfusion and thrombosis of vessels maternal decidual veins, as well as occlusive or subocclusive thrombosis of fetal great vessels of the chorionic plaque and villous trunks of the placentas of women with SARS-CoV-2[13,14]. Recently, a study by Mao et al.[15] assessed whether placental hypoxia could facilitate SARS-CoV-2 infection. The authors observed that tissue oxygenation was reduced in regions of the placenta that showed greater fibrin deposition, as in cytotrophoblastic and stroma of the chorionic villi, as well as in the cells of the extravillous trophoblast. The authors also showed that in the regions of greater fibrin deposition, the expression of the ACE2 receptor (receptor used by SARS-CoV-2 to infect the human organism) was also increased. These observations demonstrated that there was a predilection of the COVID-19 virus for regions of the placenta that are experiencing hypoxia. In these regions, ACE2 receptor expression would be increased in trophoblast cells, which would increase the chance of infection of this tissue and, consequently, the fetus.

Menter et al.[16] demonstrated that decidual microvasculopathy, manifested as signs of hypoperfusion of maternal vasculature, was a common finding in placentas of SARS-CoV-2 positive women. In addition, the authors also found a greater inflammatory response, which favors the hypothesis that SARS-CoV-2 can invade the placenta, cause an inflammatory response, and be potentially transmissible to the child, but these data were not observed in this study.

In this study, it was observed that newborns of pregnant women who had comorbidities had an increase in hospitalizations in an ICU after delivery. This result corroborates a meta-analysis that included 435 studies that observed that pregnant women positive for COVID-19 had a greater probability of having neonates admitted to the ICU after delivery when compared with pregnant women not infected by the SARS-CoV-2 virus[17].

Relationship between serological findings at delivery and placental histopathology in pregnant women with coronavirus disease 2019

In this study, we observed that patients with positive serology for IgA detected at the time of delivery presented placentas with acute fetal vascular hypoperfusion alterations disease. Dashraath et al.[6] highlighted that hormonal changes during pregnancy affect the immune response to viral pathogens. In addition, the expression of cytokines such as the interleukins (IL) IL-4 and IL-10, together with a Th2 profile and other mechanisms of immune adaptation, results in less intensity of COVID-19 symptoms in pregnant women compared with non-pregnant women[18].

Mothers with positive serology for IgA were more likely to have newborns with reactive serology for IgG at birth. These results agree with the study carried out by Gao et al.[19] who noted that among the 24 newborns of women with COVID-19, 15 (62.5%) had detectable IgG and 6 (25.0%) had IgM detectable. The RT-PCR test results were all negative. Although the levels of IgG in all 15 IgG-positive newborns have gradually decreased, they decreased more slowly in IgM-positive infants compared with those without detectable IgM. These findings reinforce the possibility of vertical transmission of the disease. Although IgG is transferred passively from the mother to the fetus through the placenta, the duration of maternal IgG passive immunity is not yet fully elucidated.

CONCLUSION

Maternal SARS-CoV-2 infection was significantly associated with adverse perinatal outcomes despite the low positivity for the virus in the placenta. Pregnant women with positive IgG at delivery had a higher incidence of placental histopathologic changes. In placentas with positive RT-PCR for SARS-CoV-2, there was a higher incidence of histopathologic changes such as maternal vascular hypoperfusion.

AUTHORS' CONTRIBUTIONS

RAMS: Conceptualization, Project administration, Supervision, Visualization. **EAP:** Conceptualization, Investigation, Methodology, Validation, Visualization. **TCC:** Data curation, Visualization, Writing – original draft. **LGCV:** Formal Analysis, Visualization. **LMÁ:** Investigation, Visualization. **EAJ:** Validation, Visualization, Writing – review & editing.

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