Vertical transmission and kidney damage in newborns whose mothers had coronavirus disease 2019 during pregnancy

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(Objective) Coronaviruses cause respiratory tract illness and further kidney damage. Here, we investigated the vertical transmission of SARS-CoV-2 from mother to child in pregnancy. The average birth weight of the 22 newborns (16 males and 6 females) was 2980 g, and the mean gestational week was 37.6 ± 3. The birth weight of three babies was < 2500 g, and the gestational week of all three low-birth-weight neonates was less than 36. Three newborns had minor lesions of infection in the lungs as shown by computed tomography (CT) scans. Furthermore, three newborns had elevated SARS-CoV-2-related immunoglobulin M (IgM) antibodies, and 11 newborns had positive immunoglobulin G (IgG) antibodies. Notably, both cystatin C and β2-microglobulin were increased in all newborns. Five of the 21 tested newborns had leukocytosis, and 11 had increased neutrophil levels. In addition, the aspartate aminotransferase of 18 newborns and the γ-glutamyl transpeptidase of 19 newborns were increased. Total bilirubin was elevated in all newborns and serum albumin was reduced in 20 of 22 newborns.

Conclusions: This study was the first to discover that COVID-19 infection in the third trimester of pregnancy could cause fetal kidney developmental injury, as indicated by increased cystatin C and β2-microglobulin in all neonates. Furthermore, there is the possibility of maternal-fetal transmission of SARS-CoV-2.

What are the novel findings of this work?

The clinical characteristics of 22 neonates born to mothers with COVID-19 indicate that COVID-19 in the third trimester of pregnancy could cause fetal kidney developmental injury and there is the possibility of maternal-fetal transmission of SARS-CoV-2.

What are the clinical implications of this work?

This study provides a theoretical basis for early diagnosis of developmental toxicity in neonates whose mothers had COVID-19 during pregnancy.

Introduction

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), broke out in Wuhan, China in December 2019. Within a few months, the
epidemic had spread around the world. As of November 10th, more than 50 million people worldwide had been diagnosed with COVID-19, which has caused over 1.26 million deaths. There is currently no validated treatment of the SARS-CoV-2, and only symptomatic supportive therapy is available for the patient. The disease is a devastating blow to all humanity. Therefore, there is an urgent need for COVID-19 clinical studies to understand the disease course to enable better control and cure of the disease.

Many clinical and basic research reports have been published on the outbreak of COVID-19. In February 2020, a study [1] published in The Lancet reported the epidemiological, clinical, laboratory, and radiological characteristics of 41 COVID-19 patients as well as the clinical outcomes of these patients. With the global outbreak of disease, more research has been conducted in other countries, such as Spain [2], India [3], and the US [4]. Correspondingly, the characteristics of COVID-19 have been gradually recognized. However, studies about COVID-19 in pregnant women and the pregnancy outcomes are still minimal, and the conclusions drawn so far are controversial. A study in The Lancet of nine pregnant women with COVID-19 found no evidence for vertical transmission [5]. Wu and colleagues [6] reported that the clinical characteristics in pregnant women with COVID-19 were similar to those of non-pregnant women.

There are studies currently exploring the issue of vertical mother-to-child transmission of SARS-CoV-2, but no consistent conclusions have been reached. For example, a case report published in JAMA [7] proposed the possibility of vertical transmission from the view of antibodies. Baud et al. reported the second-trimester miscarriage in a pregnant woman with COVID-19 that was related to placental infection with SARS-CoV-2 [8]. Although no virus was found in the fetus in this case report, the authors suggest that vertical transmission of SARS-CoV-2 warrants further investigation. A study has reported that COVID-19 placentas show an increased prevalence of maternal vascular malperfusion, which is a pattern of placental injury and is associated with adverse perinatal outcomes [9]. Penfield et al. demonstrated the presence of SARS-CoV-2 RNA in placental or membrane samples of pregnant women with COVID-19 and raised the possibility of intrapartum viral exposure [10]. The study results indicated more research is needed to confirm the possibility of vertical transmission. Kimberlin et al. proposed that the vertical transmission of the SARS-CoV-2 needs more definitive evidence, with more research required to confirm the possibility of this type of transmission [11]. However, Zeng et al. [12] found that 33% (9) neonates born to mothers with COVID-19 were identified with positive SARS-CoV2 by polymerase chain reaction (PCR), and the sources of SARS-CoV-2 in the neonates were likely maternal in origin. Hence, whether pregnant women with confirmed COVID-19 pass the virus to their offspring remains controversial and requires further study.

Therefore, to facilitate the containment of COVID-19, we retrospectively collected the data from 22 newborns of women who had COVID-19 during pregnancy.

Methods

Study design and participants

All the newborns (16 males and six females) from pregnant women with COVID-19 who were admitted to Tongji Hospital affiliated to Huazhong University of Science & Technology (Wuhan, China) from January 1 to April 1, 2020 were recruited in this retrospective study and the relevant medical indicators were recorded. Pregnant women provided verbal consent to participate in this study. All the pregnant women with COVID-19 diagnosis were based on the New Coronavirus Pneumonia Prevention and Control Program (the 7th edition) published by the National Health Commission of China. The studies involving human participants were reviewed and approved by the Ethics Committees of Tongji Hospital of Tongji Medical College, Huazhong University of Science & Technology (approval number TJ-IRB20200338).

Data collection

All the related indicators of the pregnant women with COVID-19 and the newborn were reviewed, including epidemiological history, clinical records, gestational age on infection and admission, Apgar scores, laboratory index, chest computed tomography (CT) scans, antibody information, and medical information. Amniotic fluid, umbilical cord blood, and neonatal throat swab samples were collected immediately when each baby was born. Neonatal blood samples were received within 24 h after birth for standard biochemical and blood parameter assessments, including antibody detection, blood routine examination, and blood biochemistry test. All the medical data were analysed and interpreted by two of the authors (ZH and FY). Data were recorded by each of the two authors in a particular form and the accuracy of the data checked against each other. If there was a discrepancy in the data, a third author was asked to correct it. All the COVID-19 pregnant women types were identified according to the New Coronavirus Pneumonia Prevention and Control Program (the 7th edition). All samples (including feces, urine, blood, gastric juices, and throat swab of the newborn) were tested for SARS-CoV-2 using quantitative reverse transcription PCR (qRT-PCR) with the Chinese Center for Disease Control and Prevention (CDC) recommended Kit (BioGerm, Shanghai, China), following World Health Organization (WHO) guidelines for qRT-PCR [13,14].

Sampling measurements

The latex-enhanced turbidimetric immunoassay kit (Leadman CY7600, Beijing, China) was used to test the serum cystatin C concentration on a Roche Cobas c701 (Roche, C8000-701) chemistry analyser, and it is used for clinical diagnosis (Medical Machinery Registration Certificate: 20172400748). The quantitative range of the kit was 0.2-8 mg/L. All intra-assay coefficients of variation were <5%, and the inter-assay coefficients of variation were <10%. The recommended normal range of cystatin C in the newborn is 0.63-1.25 mg/L. The latex-enhanced turbidimetric immunoassay kit (Roche 05950783, Indianapolis, USA) was used to test the serum β2-microglobulin concentration on a Roche Cobas c701 (Roche, C8000-701) chemistry analyser, which is used for clinical diagnosis (Medical Machinery Registration Certificate: 20182402206). The quantitative range of the kit was 0.1-8 mg/L. Intra-assay and inter-assay coefficients of variation were 0.8% and 1.5%, respectively. The recommended limit of β2-microglobulin is 0.2 mg/L. The chemiluminescent immunoassay iFlash SARS-CoV-2 IgG (C86095G, YHLO, Shenzhen, China) and iFlash SARS-CoV-2 IgM (C86095M, YHLO, Shenzhen, China) kits were used to test serum immunoglobulin M (IgM) and immunoglobulin G (IgG) concentration on the YHLO iFlash 3000 chemistry analyser. Intra-assay and inter-assay coefficients of variation were 10% and 12%, respectively. The recommended limit for both IgM and IgG is <10 AU/mL.

Statistical analysis

SPSS 23.0 (SPSS Science Inc., Chicago, Illinois) was used for data analysis. Continuous variables were expressed as mean±standard deviation (SD) or median (min-max) or as the number and per-
centage, as appropriate. Categorical variables were summarized as counts and percentages.

Results

Clinical characteristics of newborns of SARS-CoV-2-infected pregnant women

The clinical characteristics and the relevant detection indicators of the 22 newborns (16 males and six females) are summarized in Table 1. The average birth weight of the newborns was 2980 g, with the birth weight of only three babies (13.6%) <2500 g. Statistically, only one of the fetuses (Fetus 7) was considered intrauterine growth restriction (IUGR). The number of weeks of gestation was <36 for all three low-birth-weight neonates (35W+2, 31W, 28W+5, respectively), and the mean gestational duration was 37W+3. The mean gestational age on infection of the pregnant mother was 35W+5, which means the pregnant women gave birth within two weeks of COVID-19 diagnosis. All 22 newborns had a 1-min Apgar score of 8–9 (except Fetus 7, Apgar score of 7) and a 5-min Apgar score of 9–10 (except Fetus 7, Apgar score of 8), and there was no fetal death, neonatal death, or neonatal asphyxia observed in the study. The mean (min-max) inpatient days of the newborns was 20.05 (2-55) days. As these neonates were at high risk of SARS-CoV-2 infection, the SARS-CoV-2 nucleic acid assay was tested in the feces, urine, blood, gastric juices, and throat swab of the newborn. Fortunately, there were no SARS-CoV-2 nucleic acids in the newborns tested. Three of the newborns had minor lesions of infection in the lungs as shown by CT scans. These lesions could be the result of COVID-19 or could be aspecific lesions of other causes, such as amniotic fluid resorption delay, etc.

Laboratory characteristics of newborns of SARS-CoV-2-infected pregnant women

The laboratory indicators of the 22 neonates were collected. As shown in Table 2, the mean (min-max) cardiac troponin I (cTnI) of the newborns was 21.85 (3.1-115) pg/mL and the mean (min-max) NT-pro brain natriuretic peptide (NT-pro BNP) of the newborns was 4833.6 (611-22119) pg/mL. Blood cell count results showed that of the 21 newborns tested, five had leukocytosis, 11 had increased neutrophils, six had reduced lymphocytes, and seven had increased platelet counts. There were increased levels of aspartate aminotransferase (AST) in 18 of 22 newborns and of γ-glutamyl transpeptidase (GGT) in 19 of 22 newborns. All newborns had increased total bilirubin, 20 of 22 had reduced serum albumin, and 14 of 22 had elevated concentrations of C-reactive protein (CRP). Both cystatin C and β2-microglobulin increased in all the newborns.

Regarding SARS-CoV-2 antibodies, only three newborns (Fetus 1, 2 and 16) had elevated IgM antibodies, but 52.4% of the newborns (including Fetus 1, 2 and 16) were SARS-CoV-2 IgG-positive. Fetus 1 was delivered by cesarean section and was hospitalized for 16 days; the fetus had no other abnormal symptoms and was not treated with any drugs. IgM antibodies in the umbilical cord blood and fetal blood were 184.32 and 48.93 AU/mL, respectively. The delay between maternal infection and delivery for Fetus 1 was 41 days. The maternal symptoms were fever (the highest body temperature was 39°C) with cough and fatigue, nausea, and vomiting with diarrhea. The mother was admitted to the hospital 32 days before the delivery, and she was given symptomatic support treatment, such as oxygen inhalation therapy, prevention of infection, and promotion of fetal lung maturation. By 14 days before delivery, all symptoms of the pregnant woman had improved significantly. Fetus 2 was delivered by cesarean section and was hospitalized for 18 days; the fetus had no other abnormal symptoms and was not treated with any drugs. IgM antibody in the fetal blood was 15.75 AU/mL. The mother was diagnosed with COVID-19 by CT examination just before the delivery, and she did not have any symptoms. Fetus 16 was delivered by cesarean section and was hospitalized for 16 days; the fetus had no other abnormal symptoms and was not treated with any drugs. IgM antibody in the fetal blood was 22.8 AU/mL. The delay between maternal infection and delivery for Fetus 16 was 14 days, and she did not have any symptoms.
Table 2
Laboratory characteristics of newborns of SARS-CoV-2-infected pregnant women

<table>
<thead>
<tr>
<th>cTnl</th>
<th>NT-porBNP</th>
<th>Routine blood test</th>
<th>Liver function test</th>
<th>CRP (mg/L)</th>
<th>Renal function test</th>
<th>SARS-CoV-2 Antibodies</th>
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<tr>
<td></td>
<td></td>
<td>White blood cell count * 10^9/L</td>
<td>Neutrophil count * 10^9/L</td>
<td>Lymphocyte count * 10^9/L</td>
<td>Hemoglobin (g/L)</td>
<td>Platelet count * 10^9/L</td>
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<td>7.72</td>
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<tr>
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<td>1428</td>
<td>13.73</td>
<td>11.93</td>
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<td>3534</td>
<td>8.91</td>
<td>5.49</td>
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<td>Fetus 4</td>
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<td>4633</td>
<td>15.53</td>
<td>11.41</td>
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<td>20.66</td>
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<td>12.73</td>
<td>2.64</td>
<td>175</td>
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<td>26.2</td>
<td>3521</td>
<td>—</td>
<td>—</td>
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</table>

**cTnl:** cardiac troponin I; **NT-porBNP:** NT-pro brain natriuretic peptide; **ALT:** Alanine transaminase; **AST:** aspartate aminotransferase; **GGT:** γ-glutamyl transpeptidase; **CRP:** C-reactive protein; **IgG:** immunoglobulin G; **IgM:** immunoglobulin M

†: test value higher than normal; ‡: test value lower than normal; —: not tested
Discussion

Clinical characteristics of newborns of SARS-CoV-2-infected pregnant women

The aim of this study was to assess the clinical data from 22 neonates born between January and April 2020, whose mothers were diagnosed with COVID-19. Recent literature [15] indicates that SARS-CoV-2 infection in late pregnancy is not associated with adverse outcomes in the newborns, i.e., no asphyxia and no positive results for SARS-CoV-2 nucleic acid in the pharyngeal swab, amniotic fluid, and umbilical cord blood. Similarly, in the current study of 22 newborns, there were no deaths or neonatal asphyxia, and all the SARS-CoV-2 nucleic acid tests were negative. However, 17 of 22 newborns showed hyperbilirubinemia, which may be physiological. The average birth weight of the newborns was 2980 g. Only three babies had a low birth weight, which may be related to the <36 weeks’ gestation. Three newborns had a CT scan and were found to have minor lesions of infection in the lungs, which indicates newborns of SARS-CoV-2-infected pregnant women are at risk of COVID-19 at birth. The results of this study indicate that SARS-CoV-2 disease in late pregnancy does not affect the birth weight and SARS-CoV-2 infection status of the neonates.

Maternal-fetal transmission of SARS-CoV-2 infection

There are few reports on the influence of SARS-CoV-2 infection on newborn babies, so our understanding of this aspect is still lacking. The question of whether SARS-CoV-2 transmission can occur vertically through mother to baby has not yet been established. A few reports claim that this spread does not exist. For example, Chen et al. found no evidence for vertical transmission in nine pregnant women with COVID-19 [5]. A handful of other studies show mother-to-child transmission is unlikely for SARS-CoV-2, with the evidence for this conclusion mostly based on the fact that this virus has not been detected in fetuses [16-18]. However, these studies were inadequate as they had small sample sizes and only focused on the SARS-CoV-2 nucleic acid results. In the current cohort study, SARS-CoV-2 nucleic acids in all the newborns were negative, which is similar to the above studies. However, the current study also showed three newborns had elevated SARS-CoV-2 IgM antibody, and 11 (52.4%) of the newborns were IgG-positive at birth. As is well known, the detection of IgG antibodies in the newborns has no reliable value as these antibodies could have transferred from the mothers. Although IgM antibodies could reflect a fetal immune reaction, some cross-reactions have been described between the antibodies of COVID-19 and others. Recent studies showed there were cross-reactions between rheumatoid factor and the IgM of SARS-CoV-2, leading to false-positive results [19]. Moreover, the intra-assay and inter-assay coefficients of variation were 10% and 12% for IgM detection, so there may have been cross-reaction. Placental alterations may allow the passage of IgM. The study by Nakamura et al. indicates that IgM was transported from the maternal tissues to embryos via a unique pathway in the viviparous teleost [20]. None of the newborns in the current study were positive for SARS-CoV2 nucleic acids, so the elevated SARS-CoV2 IgM level in the three newborns at birth indicate there was very probably an intratuterine infection of the SARS-CoV-2. Interestingly, the umbilical cord blood IgM for one of the fetuses (Fetus 1) was positive (184.32 AU/mL). Antibody tests were not performed in the umbilical cord blood of the remaining 21 fetuses. We speculate the possibility of maternal-fetal transmission of the virus, which is similar to the study published by Zeng et al. [12].

Kidney damage in the newborn of SARS-CoV-2-infected pregnant women

Most research shows no symptomatic neonates born to mothers with confirmed COVID-19 [5,16]. Li et al. found mothers with COVID-19 did not have associated severe neonatal complications [21]. However, in the current study, mean Ctnl of the newborn was 21.85 pg/mL, and mean NT-pro BNP was 4833.6 pg/mL. In addition, blood cell count results showed that of the 21 newborns tested, five had leukocytosis, and 11 had increased neutrophils. Furthermore, liver function test results showed that AST was increased in 18 of 22 newborns, all newborns had increased total bilirubin, and 20 of 22 had reduced serum albumin. Some studies indicated that COVID-19 patients showed elevated GGT levels [22,23]. A meta-analysis analysed 128 COVID-19 studies and found that the most frequent abnormality in liver functions was hyperglobulinemia, followed by disorders in GGT [24]. In the current study, GGT was increased in 19 of 22 newborns. These results indicate the possibility of neonatal liver damage. However, some of the neonates had hyperbilirubinemia, which does not resolve physiological jaundice.

As a potential biomarker for neonatal renal function, cystatin C and β2-microglobulin significantly improved the risk classification for death and renal disease across diverse populations [25,26]. Elevation of cystatin C occurs before the increase in serum creatinine. Some pediatric studies showed that cystatin C level was potentially better measure than serum creatinine for estimating renal function in newborns [27,28], and a better marker for estimating glomerular filtration in preterm infants [29]. Bokemamp et al. found that cystatin C and β2-microglobulin concentrations in fetal serum would be useful predictors for postnatal kidney function [30]. Notably, both cystatin C and β2-microglobulin increased in all the newborns, which was not reported in previous studies. The above results indicate that confirmed COVID-19 in pregnant women may lead to fetal kidney damage.

Strengths and Limitations

Although the results of the current study indicate the possibility of vertical transmission between mother and child, the small sample size means more research is needed to confirm this conclusion. In this study, virus (IgM) antibody was detected in the umbilical cord of only one fetus; antibody tests were not performed in the umbilical cord of the remaining 21 fetuses. Fetal serum for all fetuses was tested for SARS-CoV-2 antibodies; IgM was positive for only three newborns. So far, only Zeng et al. [12] found that 3 of 33 (9%) neonates born to mothers with COVID-19 were identified with positive SARS-CoV2 by PCR, and the sources of SARS-CoV-2 in the neonates were likely maternal in origin. This is why vertical propagation can only be suggested and more studies are needed to confirm this conclusion. Most researchers are focusing on the presence of COVID-19 nucleic acids in the neonates. When the results were negative, they concluded that there was no mother-to-child transmission. This is not reliable because infectious diseases are inherently self-healing. Therefore, follow-up studies are required to verify this conclusion in more samples and with more time points. Maternal COVID-19 causing kidney damage in neonates has not been reported before. Follow-up studies are needed to explore whether the impact is long-term.

Conclusions

The clinical characteristics of the newborns in this study indicate that SARS-CoV-2-infected pregnant women have a favorable pregnancy outcome, and SARS-CoV-2 infection in the late stage of pregnancy does not affect the fetal birth weight or SARS-CoV-2 infection status. There is the possibility of maternal-fetal transmis-
sion of the SARS-CoV-2 virus, due to the presence of COVID-19 IgM antibody in umbilical cord blood and fetal blood. Early kidney damage, as shown by increased cystatin C and β2-microglobulin, was found in all the newborns in the study. The authors propose the possibility of vertical mother-to-child transmission of the SARS-CoV-2 virus and the resulting renal damage, which is vital for understanding the clinical characteristics of newborns of pregnant women with COVID-19. Although the results of this study should be evaluated with caution because of the small number of newborns, they increase the awareness of the potential effects of SARS-CoV-2 on newborns. This study provides some clinical support for the early warning of SARS-CoV-2 damage on neonates.

Author contributions

ZH, YF, and QZ contributed to the acquisition of patient data. ZH and XH performed the study design, interpretation and manuscript writing. ZH and YL assisted with data analysis and statistical methods. YL, XR and DL provided critical revision of the manuscript for important intellectual content.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

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Competing Interests: No conflicts of interest are declared by the authors.

Ethical Approval: The studies involving human participants were reviewed and approved by the Ethics Committees of Tongji Hospital of Tongji Medical College, Huazhong University of Science & Technology (approval number TJ-IRB20200338).

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