Follow-up study on the outcomes of recovered pregnant women who had previous COVID-19 during the first and second trimester: a retrospective cohort study

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Abstract

Objective: The research aimed to study the pregnancy outcomes and determine the safety of continued pregnancy until the third trimester after being infected with SARS-CoV-2 during early pregnancy. Design:Retrospective cohort study. Setting: Wuhan, Hubei, China.Population:Pregnant women in recovery who had a history of COVID-19 during early pregnancy at the Union Hospital from 15 Jan 2020 to 30 April 2020 were included. Methods:The clinical manifestation, laboratory examinations, treatment, pregnancy outcomes, maternal SARS-CoV-2 throat swab RT-PCR test results, and SARS-CoV-2 antibody tests in neonates were detected. The placental pathology, ACE2 expression and SARS-CoV-2 presence was also studied. Main outcome measures: Maternal and neonatal clinical and laboratory status. Results: We included cases in our study. Three cases were diagnosed with COVID-19 during early pregnancy (Case A to C), and two cases were serum IgG positive asymptomatic cases (Case D and E). Case A showed complete recovery after severe COVID-19. Case C was infected at 6 weeks during the first trimester and had induced medical abortion at 12 weeks. No pneumonia was found in all neonates, the nucleic acid test and serum IgM were negative, and IgG were positive. All placental samples were negative for SARS-CoV-2 nucleic acid test. Infants followed-up at 1-3 months were healthy and asymptomatic. Placental pathology showed chronic ischemia performance.Conclusions COVID-19 during pregnancy did not significantly affect pregnancy outcomes. No adverse outcomes were observed in all the neonates. The inflammatory state of COVID-19 may cause placental injury. The placenta does not appear be a target organ for SARS-CoV-2.

Tweetable abstract:

Pregnant women with COVID-19 during early pregnancy were assessed to determine the pregnancy outcomes.

INTRODUCTION

The novel coronavirus infection (COVID-19) was prevalent in Wuhan since December 2019, including pregnant women[1]. In Wuhan, these patients represented 0.24% of all reported cases with COVID-19[2]. COVID-19 affects the entire pregnancy; however, current research is mostly about pregnancy during the third trimester and peripartum[2-4]. In New York, of the 215 women screened for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on admission using nasopharyngeal swabs, 33 women (15.4%) were found positive. Of these positive cases, four had fever or other symptoms of COVID-19, while 29 were asymptomatic[3].

Currently, there are a few reports on the outcome of COVID-19 in pregnant women during the first and second

trimester, as well post-recovery. The effect of COVID-19 on pregnancy remains unclear, which obstetricians and neonatologists. Recent researches indicated that there is no clear evidence of intrauterine infection in pregnancy with COVID-19 during the third trimester[2,5]. However, both SARS-CoV-2 IgM and IgG were detected in newborns of infected mothers, which question the possibility of intrauterine infection[6,7]. Some studies claimed that a positive IgM test result could not be regarded as the definite evidence of vertical transmission[8]. Our previous study reported the clinical characteristics and placental pathological analysis of three peripartum women with COVID-19[9]. We noted no severe adverse pregnancy outcomes or morphological changes related to infection. All samples were negative for the SARS-CoV-2 nucleic acid[9]. We concluded that COVID-19 intrauterine infection does not occur during the third trimester.

The aim of our current study is to determine the safety and postpartum outcomes in pregnant patients infected from early pregnancy (first and/or second trimester) with COVID-19.

METHODS

Study design and participants

This is a retrospective cohort study done at Union Hospital, China from 15 Jan 2020 to 30 April 2020. A total of five cases were included in this study. This study included three fully-recovered pregnant patients confirmed with COVID-19 during the first or second trimester (<28 weeks gestation). COVID-19 recovery criteria were defined according to the World Health Organization interim guidance criteria[10] and the Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and Treatment[11], which are as follows: (1) normal or significantly improved chest computed tomography (CT), (2) negative SARS-CoV-2 nucleic acid test, and (3) negative SARS-CoV-2 serum IgM, and positive or negative IgG. Two asymptomatic cases with positive IgG at the time of delivery were also included. Case D and case E developed mild cold at 26 week and 27 weeks, they did not seek medical attention. The symptoms improved after 1 week. Clinical symptoms of COVID-19, laboratory results, CT imaging results, treatment course, pregnancy outcomes, placental pathology, SARS-CoV-2 laboratory results, angiotensin-converting enzyme 2 (ACE2) expression, and neonatal throat swabs and antibody tests were analysed.

We reviewed the medical records, laboratory results, and imaging findings of the recruited cases from admission to postpartum. Laboratory examination before admission includes SARS-CoV-2 pharyngeal swab and serum antibody, and chest CT scan. Postpartum, neonatal throat swab, umbilical cord blood antibody testing, and QT-PCR for SARS-CoV-2, and placental pathology were performed. Laboratory confirmation of SARS-CoV-2 infection was done. SARS-CoV-2 infection was confirmed using real-time RT-PCR[12].

This study was approved by the Medical Ethics Committee of the Union Hospital, TongJi Medical College of HuaZhong University of Science and Technology [2020, NO. 0144]. Written informed consent was obtained from the patients before enrolment and during data collection.

The Serum antibody test: SARS-CoV-2 IgG and IgM Colloidal gold method (Vazyme, NanJing, China) on 20 μml plasma.

Pathological examination: Placental tissue specimens (Case A, B, C, D) were fixed using 3.7% formaldehyde solution for 48 hours. Conventional HE staining was performed after paraffin embedding, each slice was 4μm thick. All HE sections were observed and reviewed by one attending physician and one associate chief physician. Using RT-PCR method to detect SARS-CoV-2 in placental tissue: three wax blocks were selected for SARS-CoV-2 detection in each case, and the samples to be tested included the placental villi (including maternal and foetal surfaces), foetal membrane and umbilical cord as far as possible. Formaldehyde-fixed paraffin-embedded sample RNA separation kit and SARS-CoV-2 detection kit were used (Aide biological medicine technology co., Ltd, XiaMen, China). ACE2 Recombinant rabbit monoclonal antibody (Clone No. BP6153, Bailing (Biolynx) Biotechnology Co., Ltd. Hangzhou, China) was used to study the expression of ACE2 in the placenta.

ACE2 evaluation criteria: the area quartering method was 1, 2, 3, and 4 points. The staining intensity was 1 (weak), 2 (medium), and 3 (strong), respectively. The total score was multiplied by each other.

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RESULTS

Early pregnancy infections were noted in three cases who were infected at 24 and 26 weeks and recovered after treatment for pneumonia. Before delivery, CT imaging showed resolution of pneumonia. Throat swab SARS-CoV-2 tests were done twice with negative results. Only the SARS-CoV-2 IgG antibody was positive. Case A delivered at 40 weeks age of gestation after administration of dinoprostone suppository improving cervical ripening and oxytocin augmentation of uterine contraction. Case B had premature rupture of membranes at 35 weeks with transverse presentation and delivered via caesarean section. Case C was infected at 6 weeks who underwent an induced medical abortion at 12 weeks after administration of mifepristone and misoprostol.

Additionally, two COVID-19 asymptomatic cases were (Case D and E) who had serum SARS-CoV-2 IgG positive on routine screening before delivery, chest CT scan and nucleic acid tests were unremarkable.

COVID-19 during pregnancy

Following the WHO interim guidance criteria, three cases were diagnosed with COVID-19: Case A was 24 weeks (+3), while Case B was 26 weeks (+1) when they were diagnosed with COVID-19. They had no chronic disease. Both presented with fever, fatigue, and dry cough. The descriptions are listed in Table 1. Case A had respiratory distress and dyspnoea; Case B presented with stuffy nose and respiratory distress. Case C had no dyspnoea. All three cases had moderate anaemia. Case B had lymphopenia (0.54 G/L), leukopenia (3.21 G/L), and neutropenia (1.65 G/L). Case C presented with moderate leukopenia. Cases A, B, and C had elevated C-reactive protein and IL-6 (Case A:CRP 6.9ng/L, IL-6 67.98 pg/ml,Case B CRP 31.88mg/L, IL-6 not available; Case C: CRP 26.8 ng/L, 7.49 pg/ml); and had decreased albumin and total protein (TP). Case A (TP 55.8 g/L, ALB 27.9 g/L , ALT 510 U/L, AST 246U/L) , Case B (TP 57.1 g/L, ALB:31.4 g/L, AST 21 U/L, ALT 14 U/L) , Case C: (TP: 57.6 g/L, ALB:31.4 g/L, ALT 38U/L, AST 104U/L). Case A and C had positive nucleic acid tests. Case B had two consecutive negative nucleic acid tests, however, she presented with viral pneumonia. On readmission after 70 days, SARS-CoV-2 IgG was positive confirming previous COVID-19 infection. Case A had a severe type of infection and was admitted for 12 days. Case B and C were normal type infections and were treated for 6 days and 10 days, respectively. According to chest CT imaging, they all had viral pneumonia. CT imaging of Case A showed multiple, membranous, ground-glass-patterns or dense shadows surrounding the parenchyma of both lungs. The pulmonary lesions showed resolution post-treatment. Case B and C show similar chest CT findings (Fig. 1).

All cases received oxygen therapy using a nasal catheter. Case A had severe dyspnoea, fatigue, and desaturations (SPO_2 90% on room air). Case B and C had moderate distress ($SPO_2$95-96%) (Table 1).

Case A received antiviral treatment, interferon (Recombinant human interferon a-2b 5 million IU daily), atomisation inhalation for 7 days, oral umifenovir tablets (200 mg three times daily for 7 days), and azithromycin (0.25g bid for 3 days). Polyene phosphatidyl choline (458 mg thrice daily) and reduced glutathione (1.8 g daily) were used to decrease the ALT and AST for 7 days. All cases were given antibiotic treatment (Table 2.) They were discharged improved and stable. CT scan showed resolution of pulmonary lesions, and the SARS-CoV-2 throat swab showed two consecutive negative results. After discharge, Case A and C were prescribed oral liver supplements and was quarantined for 14 days. Repeat antibody testing before delivery showed positive serum IgG and negative IgM antibodies in all three cases (see Table 2).

Pregnancy outcome

The interval period from discharge to delivery was 42 to 109 days for all three cases. Case A was admitted at 40 weeks, with a cervical score of 6. After dinoprostone administration and oxytocin, a 3650 g male was delivered with an APGAR score of 8 at 1 minute and 9 at 5 minutes. Epidural labour analgesia was

administered when the cervix 3 cm dilated. Case B had premature rupture of membranes at 35 weeks with transverse presentation delivered using caesarean section to a 2800 g female baby. APGAR score was 7 at 1 minute and 9 at 5 minutes.

The newborns of Case A and B had no pneumonia, negative COVID-19 throat swab nucleic acid test, and positive serum IgG. Neonate A was roomed-in and was breastfed. At 1 month follow-up, neonate A was healthy and asymptomatic. Neonate B was transferred to NICU due to preterm labour. The neonate remained asymptomatic during admission and was discharged after a week following two consecutive negative nucleic acid test results. IgG was positive. At 2 months follow-up, the infant was healthy and mixed-feeding.

The neonates of Case D and E were healthy and asymptomatic. Both of them showed negative SARS-CoV-2 throat swab nucleic acid test, and positive serum IgG (Table 2).

Placental examination

The placental tissues of Case A to D were examined which showed varying degrees of injury. Case A showed multiple placental calcifications and increased syncytiotrophoblast knot. Case B showed placental chronic infarct at the maternal side. The villous structure collapsed with fibrin deposition. The middle of the placenta was normal. Case C showed oedema in the villi and fibrosis in the 12-week-old placenta. No definite thrombosis was found. No obvious chorioamnionitis or virus inclusion body formation was observed in all cases. The umbilical cord and amnion membranes were normal (Fig.2).

Immunohistochemical staining of ACE2 showed that both the placenta and the decidual membrane expressed ACE2, which was located in the membrane and cytoplasm of the syncytiotrophoblast and decidual cells. The placental expression was strongest at 12 weeks of gestation (Fig. 3).

Placental SARS-CoV-2 nucleic acid test results of all the cases Case A,B,C,D were negative (Table 1).

DISCUSSION

Main Findings

The current follow-up study showed that, pregnant women infected with SARS-CoV-2 in the early pregnancy and recovered had good outcome. No pneumonia was found in all neonates, the nucleic acid test and serum IgM were negative, and IgG were positive. All placental samples were negative for SARS-CoV-2 nucleic acid test. Placental pathology showed chronic ischemia performance.

Strengths and Limitations

Our study has several strengths. First, it was a retrospective cohort study allowing us to evaluate the outcomes of COVID-19 from early pregnancy to follow-up. Second, we have evaluated several parameters during early pregnancy including clinical, laboratory, imaging and placental pathology.

Meanwhile, our study has some limitations. Our study only enrolled five cases with COVID-19. Therefore, our results may not be generalizable for other populations. The number of cases is limited and further studies are needed. Second, foetal and maternal outcomes were only observed until two-months of follow-up, which provides limited information. Further follow-up is important to assess the long-term effects of maternal COVID-19 to the infant.

Interpretation

According to the data of 118 cases of COVID-19 pregnancy in Wuhan[2], 64% of the cases occurred during late pregnancy, and the rate of severe disease in all patients with COVID-19 pregnancy was 8%, which was lower than the average level of the general population (15.7%). There were no deaths reported. It has been suggested that pregnancy might confer potential immunity. This was consistent with our study wherein even severe COVID-19 improved after proper treatment (Case A).

Among the three confirmed patients (Case A, B, C), only two (Case A and C) showed elevated liver enzymes possibly because of COVID-19-related hypoxia augmenting the inflammatory factors. Additionally, we found

a pro-inflammatory state in COVID-19 (elevated CRP and IL-6 levels). The liver was also affected as seen from the abnormal liver function tests. Finally, after the administration of antiviral therapy (umifenovir and interferon atomisation) and liver supplements,liver function profile improved.

None of the patients showed significant foetal malformation or foetal or placental virus infection. Even Case C, which was prematurely terminated, had crown-lump length (CRL) at 12 weeks at par with age.

Placental pathology suggest chronic placental injury, hence has the possibility of decreasing maternal blood perfusion into the villi, thus, hindering maternal-foetal exchange[2]. This may be secondary to the hypoxic and inflammatory condition during COVID-19 affecting the placenta. Massive perivillous fibrin deposition and large agglomeration of necrotic villi suggested placental infarction was also found in Case B. The preterm premature rupture of membrane in Case B may be not only due to transverse presentation but also associated with the chronic ischemia and increased cytokines. Fortunately, only significant placental infarction was associated with SGA foetuses[13]. In the present study, although placental damaged was noted, the outcomes of were relatively ideal. The placental potency may be enough to overcome the villi impairment. However, if a mother had some complications associated with placental dysfunction (preeclampsia, fetal growth retardation, et al), COVID-19 may severely affect the placenta and foetus and result to adverse pregnancy outcome. These studies indicated that pregnancy with COVID-19 during the first and second trimester may result to full recovery allowing the continuation of pregnancy to term with good maternal and foetal outcomes.

All the placenta tests were negative for SARS-CoV-2 nucleic acid. In our previous study, same negative results were found in the three cases who had COVID-19 just before delivery[9]. The recent study cannot provide definite evidence to support intrauterine infection with COVID-19.

Vertical transmission includes intrauterine infection during pregnancy, intrapartum infection, and transmission through close contact and breastfeeding after delivery. ACE2 is a functional receptor for SARS-CoV-2 infection[14]. Theoretically, ACE2 provides the opportunity for SARS-CoV-2 infection. SARS-CoV-2 attaches to the cells using S protein and enter cells through binding to ACE2. It was suggested that the distribution and expression of ACE2 may be critical for the target organs affected by SARS-CoV-2[15,16]. The available evidence suggests that ACE2 is widely expressed in female reproductive system and the maternal-foetal interface, which includes the stromal cells and perivascular cells of decidua, cytotrophoblasts and syncytiotrophoblasts[14,17]. Our results showed that both the placenta and decidua expressed ACE2 during early pregnancy with significantly higher levels than those during late pregnancy. However, the histologic and real-time PCR of the placenta had the negative results. Therefore, organs with high ACE2 expression may not always be the target of SARS-CoV-2.

During vertical transmission, high viral loads of SARS-CoV-2 is necessary. The placental villi may have more opportunities for SARS-CoV-2 to enter the maternal blood allowing the virus to infect the trophoblast cells. SARS-CoV-2 may then pass through the placental villous lobules to enter the foetal capillaries and infect the foetus. According to some reports, the lungs and the intestine may be major viral target organs of SARS-CoV-2[18]. Studies have shown a high expression of ACE2 and TMPRSS2 in the lungs of newborns[19]. This places neonates at high risk for SARS-CoV-2 infection.

A Lamouroux et al[20] reported three swabs positive for SARS-CoV-2 in 11 placental or membrane swabs sent following delivery in women with moderate to severe COVID-19. None of the infants tested positive for SARS-CoV-2 on days 1 to 5 of life, and none demonstrated COVID-19 symptoms. While there were no clinical signs of vertical transmission, they postulated on possible intrapartum viral exposure. For infants delivered vaginally, viral contamination may be from room air, vaginal secretions, maternal blood, or amniotic fluid. In our study, no virus or viral infection was found in the placenta. Wang et al[21] reported a case of positive SARS-CoV-2 throat swab test in a newborn 36 hours after delivery. Airborne transmission possibility after delivery cannot be excluded. The risk of neonatal infection from postpartum exposure via respiratory secretions and close contact should be avoided. The neonate throat swab tests and placenta tests were negative in our cases. This reminds obstetricians that although there is no clear evidence of intrauterine infection, careful attention to protect the neonate from exposure to SARS-CoV-2 during and after delivery

should be observed.

In clinical studies, most of the neonates were negative of SARS-CoV-2[2,4]. The placenta may not be the main target organ of SARS-CoV-2. There is no clear evidence of intrauterine infection, which requires further study regarding the specific mechanisms involved.

CONCLUSION

Pregnancy complicated with COVID-19 does not show significant effects on pregnancy outcomes. No adverse foetal outcomes were observed during the neonatal follow-up (until 3 months) in the infants born to mothers infected with COVID-19 during early pregnancy. COVID-19 during pregnancy may lead to a systemic inflammatory response and hypoxia, resulting in elevated liver enzymes and placental insufficiency. The placenta has a potential reserve capacity allowing pregnancy to continue to term. Although the placenta and decidua express ACE2, the placenta does not appear be a target organ for SARS-CoV-2. Possibly, the placental barrier can effectively prevent SARS-CoV-2 from causing intrauterine infection.

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Contribution to Authorship:

ZY and ZL conceived the study. HBX and NX performed placental study. MH carried out the CT image data collection. SY collected the clinical data. ZY wrote the first draft. NX and ZL reviewed the article.

Details of Ethics Approval:

The study was approved by the Medical Ethics Committee of the Union Hospital, TongJi Medical College of HuaZhong University of Science and Technology [2020, NO. 0144]. Written informed consent was obtained from the patients before enrolment and when data were collected.

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Figure Legend

Fig 1. Chest CT image of the cases.

A1, B1, C1 were the CT scanning images of pregnant women complicated with COVID-19. Multiple or scattered membranous glass density shadows/dense porphyry shadows surrounding the parenchyma of both lungs could be seen at the onset of Covid-19, which indicated the possibility of viral pneumonia is high. A2,C2 were the CT image which showed lesions in lung were improved after being treated for 7 days. A3, B2, C3 were the CT image at the time before delivery, no obvious abnormality was observed.

Fig 2. Pathological findings of placental tissue

Case A : Pregnant woman of 24weeks complicated with Covid-19, and delivered at 40 weeks. Placental chronic ischemia was shown : presence of multiple placental calcifications (black arrow) and dense small villi with increased syncytial knots (blue arrow) . (1: HE x40, 2: x100)

Case B: Pregnant woman of 26 weeks complicated with COVID-19, and delivered at 35 weeks. Placental chronic infarct was seen at the maternal side (40 times magnification) . In the middle part of the placenta, normal villi structure could be seen. (100 times magnification).

Case C: Pregnant woman of 6 weeks complicated with COVID-19, and the pregnancy was terminated at 12 weeks. Part of the villous was edema and fibrosis (Arrow) (40 times)." No definite thrombosis was found.

Case D: Pregnant women of 38 weeks who was serum IgG positive asymptomatic case. Chronic placental ischemia infarction could be seen. (1: HE x40, 2: x100).

Fig 3. Representative ACE2 Immunohistochemical staining in placenta.

ACE2 was positive in both villi and decidua, which was mainly expressed in membrane and cytoplasm. It expressed stronger in 12 weeks (Case C) (x 100 times). ACE2 evaluation criteria: the area quartering method was 1, 2, 3 and 4 scores, and the staining intensity was 1 (weak), 2 (medium) and 3 (strong), respectively. The total scores were area scores multiply staining intensity scores.

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