Title: Double Jeopardy: The Intersection of COVID-19 and Pregnancy in an Educational Hospital, Northern Iran, Gorgan

Running title: COVID-19 in pregnant women

Abstract

With the onset of the severe acute respiratory syndrome coronavirus-2 pandemic, there were controversial theories regarding the potential consequences of the virus on pregnant women and delivery outcomes. During the past three years, a great diversity of literature reported various data regarding covid-infected mothers and pregnancy-related complications including preterm birth, stillbirth, preeclampsia, cesarean delivery, etc., however; the exact influences which can be exerted by the virus and possibility of vertical transmission, still remained obscure. Here we described the clinical features and outcomes of delivery in 16 laboratory confirmed COVID-19-infected mothers referring to a hospital in northern Iran from August 2020 to December 2021. Clinical records, laboratory results, and chest CT scans in addition to such samples as maternal peripheral blood, umbilical cord blood, placental blood, vaginal secretion, placental tissue, breast milk after first lactation, and neonatal throat swab and peripheral blood were collected to answer the questions raised on the possibility of vertical transmission of COVID-19 and transferring maternal immunity to the neonates, all the aforementioned specimen were evaluated based on molecular and serological assays. SARS-CoV-2 RNA was not detected in vaginal secretions and placental tissue. SARS-CoV-2 IgG and IgM antibodies were detected in 15 and 4 maternal blood samples, respectively; in one breast milk sample (IgM), two umbilical cord blood (IgG) samples, two placental blood (IgG) and two neonatal blood (IgG) samples. Chest CT scan of abnormal cases revealed typical signs of viral pneumonia. According to the current study there seems to be

associations between SARS-CoV-2 infection and the risk of preterm birth; however, no intrauterine vertical transmission of SARS-CoV-2 was found. These results also suggest the possibility of passive IgG transfer from the infected mothers to their neonates.

Keywords:
COVID-19, Pregnancy, Infectious Disease Transmission, Vertical, Iran.

1. Introduction

SARS-CoV-2, the virus responsible for COVID-19, has had notable effects on pregnant women. According to several studies (1), the consequences of SARS-CoV-2 infection during pregnancy can be significant. It may lead to an increased risk of complications, such as preterm birth, preeclampsia, and severe respiratory issues in expectant mothers. Additionally, research has shown a potential for vertical transmission, where the virus can be passed from the mother to the fetus, although this occurrence is relatively rare. Therefore, it is crucial for pregnant women to take precautions and follow recommended guidelines to minimize the risk of exposure to the virus and its potential adverse effects on both their health and that of their unborn child (2).

Certain viral respiratory outbreaks, including SARS-CoV, MERS, and 2009 Influenza A (H1N1), have been known to induce detrimental effects on pregnancy status. These negative impacts encompass a higher risk of adverse outcomes such as preterm birth, stillbirth, maternal mortality, severe respiratory distress, pneumonia, acute respiratory distress syndrome (ARDS), and an elevated likelihood of complications affecting both maternal and fetal health (3, 4).

During the past five years, literatures reported various data regarding COVID-19-infected mothers and such pregnancy-related complications as preterm birth (PTB), stillbirth, preeclampsia, cesarean delivery. However, the exact pathogenesis including possibility of vertical transmission

still remains obscure (5). Additionally, another question is the possibility of maternal SARS-CoV-2 antibody transfer to the fetus or neonate before birth or during the lactation period. Furthermore, there aren't proved explanations of exact clinical characteristics of COVID-19 in both the mother and her neonate (6).

Here we described the clinical features and pregnancy outcomes in 16 laboratory confirmed COVID-19-infected mothers referring to a hospital in northern Iran from August 2020 to November 2021.

2. Methods

2.1.Study population and clinical samples

The population of interest for the current study consisted of pregnant women with laboratory-confirmed COVID-19 referring to the maternity ward of Sayyad Hospital, Northern Iran due to delivery. Women were recruited from August 2020 to November 2021.

2.2.Data collection

The study's inclusion criteria required pregnancy and a laboratory-confirmed COVID-19 test at any point during pregnancy. The exclusion criteria were non-pregnancy and a negative COVID-19 test. Sterile Dacron swabs with flexible plastic shafts were used to collect maternal and neonatal throat and nasopharyngeal swabs immediately after admission and birth, respectively, and tested for SARS-CoV-2 using the Iranian Center for Disease Control and Prevention (CDC) recommended Kit (PISHTAZ TEB COVID-19 One-Step RT-PCR Kit Dual-target gene (nucleocapsid protein (N) and RNA-dependent RNA polymerase (RdRp)) following WHO guidelines for qRT-PCR. All samples were processed simultaneously at the Department of Microbiology, School of Medicine, Gorgan University of Medical Sciences, Gorgan, Iran. Maternal peripheral blood samples were taken and sera were aliquoted after centrifugation. SARS-CoV-2 IgG and IgM antibodies were assessed in sera using IDEAL TASHKHIS IgG and IgM ELISA kits. Umbilical cord blood, placental blood, vaginal secretions, placental tissue, and neonatal throat swabs and peripheral blood samples were collected immediately after delivery in the isolated negative-pressure operating room. Additionally, breast milk samples from patients with COVID-19 were collected after their first lactation. All aforementioned specimen were tested

using qRT-PCR and serological assays. The clinical records and chest CT scans of all patients were meticulously examined to extract relevant data.

3. Results

3.1.Clinical features of mothers with COVID-19 infection

In a 16-month study from August 2020 to November 2021, 16 third-trimester pregnant women were hospitalized. Their clinical and lab data and treatments are summarized in Table 1. Ten underwent caesarean sections and six vaginal deliveries. Patients' ages ranged from 21 to 38 years, and their gestational ages at delivery were between 26 weeks and 4 days and 38 weeks and 5 days. BMI $\geq$ 30 kg/m2 was reported in 7 cases. No underlying diseases, except for one with cardiovascular disease, were detected, and 7 patients developed gestational diabetes, while only one had preeclampsia.

In 10 out of the 16 patients, fever was reported before or during delivery, with body temperatures ranging from 36.5°C to 38.0°C. Two patients experienced postpartum fever, with temperatures ranging from 37°C to 39°C. Moreover, other symptoms of upper respiratory tract infection were reported: 11 patients had a cough, seven reported myalgia, and sore throat and chest pain was observed in two and three women, respectively. Seven women indicated dyspnea and two reported headaches. In none of the patients, gastrointestinal symptoms were observed. None of the patients exhibited gastrointestinal symptoms, and none required mechanical ventilation or died from COVID-19 pneumonia. Three patients were admitted to the ICU, and six and five patients used nasal cannula and nonrebreather mask for oxygen support, respectively. Nine patients received antiviral therapy (table 1).

The clinical parameters, such as lymphopenia ($<1{\cdot}0 \times 10^9$ cells per L) and elevated levels of C-reactive protein (>10 mg/L), were extracted from medical records (table 2). In five cases, elevated concentrations of alanine aminotransferase (ALT) or Aspartate aminotransferase (AST) were reported, and in four patients, increased white cell counts (>11.0 × 109/L) were observed.

The results of the SARS-CoV-2 qRT-RNA and anti-SARS-CoV-2 antibody ELISAs for 16 patients are summarized in Table 2. All COVID-positive tests were confirmed by both nasopharyngeal and oropharyngeal qRT-PCR. SARS-CoV-2 RNA was not detected in vaginal secretions or placental tissue. Serological tests performed on mothers' blood (15 IgG positive and

4 IgM positive), breast milk (one IgM positive), umbilical cord blood (two IgG positive) and placental blood (two IgG positive) (Table 2).

Chest CT scans were performed on all 16 patients, but we only accessed 8 scans, which in abnormal cases showed typical signs of viral pneumonia such as decreased diffuse and bilateral ground-glass opacities, patchy lung consolidation, blurred borders, and lesions merged into strips (Figure 1).

3.2.Clinical characteristics of neonates born to mothers with COVID-19 infection

Delivery occurred in an isolated room, and the infants were immediately separated from their mothers. Among the infants, ten were born full-term and six were premature. A total of 16 live births were recorded, with two fatal distress cases. Three newborns passed away after delivery. Eight infants had a birth weight of less than 2500g, and three of them did not survive. The 8-10, 1-, and 5-min Apgar scores were recorded for 13 newborns, while three neonates who finally passed away had 5-6 and 7-8, 1-, and 5-min Apgar scores. Pneumonia and pneumothorax were observed in seven and three newborns, respectively. Four neonates required mechanical ventilation, and nine required oxygen therapy. Two newborns had Respiratory Distress Syndrome (RDS), one had intraventricular hemorrhage (IVH), and six had sepsis. According to CDC recommended qRT-PCR test, SARS-CoV-2 nucleic acid was not observed in any of cases. Furthermore, Anti SARS-CoV-2 IgG was detected in blood samples of two newborns (Table 3).

4. Discussion

Pregnancy triggers physiological adjustments to ensure optimal fetal development and delivery outcomes. However, these adjustments also make mothers more susceptible to pathogens. The potential for neonatal morbidity and developmental malformations resulting from viral infections during pregnancy highlights the importance of understanding the likelihood of vertical transmission of SARS-CoV-2 (4). Here, none of the newborns' nasopharyngeal swabs tested positive for the virus, and the molecular tests of umbilical cord blood, placental blood, vaginal secretion, placental tissue, and neonatal peripheral blood were also negative, consistent with the results of previous studies (7). While some studies suggest evidence of vertical transmission of SARS-CoV-2, mostly through positive neonatal throat swab tests , which may be more indicative of postnatal transmission, data showing SARS-CoV-2 RNA in placenta, amniotic fluid, and

umbilical cord blood supports the hypothesis of vertical transmission, albeit with a rare occurrence (8). The expression of angiotensin-converting enzyme 2 (ACE2), trans-membrane protease serine 2 (TMPRSS2), dipeptidyl peptidase 4 (CD26), and CD147 in syncytiotrophoblast (SCT), villous (VCT), and extravillous (ECT) cytotrophoblast, as well as gynecological organs like the vagina and ovary, which are involved in viral entry may explain these results; however, the findings in the literature are controversial (9).

The study analyzed the clinical characteristics of SARS-CoV-2 infected mothers and their newborns, revealing that fever, cough, myalgia, dyspnea, sore throat, and gastrointestinal symptoms are common among them, which aligns with other studies and show that these symptoms are not unique to SARS-CoV-2 infected pregnant women (10). The literature review revealed mixed results on the link between COVID-19 and preeclampsia (PE). Some studies found associations between the two factors (11), while others did not. It is important to note that these studies referred to a temporary condition called preeclampsia-like syndrome (PE-like syndrome), which shares symptoms with PE and makes it difficult to distinguish between the two conditions (12). Our research also did not uncover any associations between COVID-19 and PE. The survey then focused on the relationship between COVID-19 and obesity. Apart from the Center for Disease Control and Prevention (CDC), which identified obesity as a high-risk group for severe illness and death from SARS-CoV-2, previous research has also found links between this condition during pregnancy and adverse outcomes from various infections, including CMV, documented influenza pandemics, Varicella Zoster, malaria, Listeria monocytogenes, and SARS. This correlation can be attributed to leptin, an adipocyte-derived hormone that plays a role in food intake, reproduction, and immunometabolism, and is linked to inflammatory pathways that can exacerbate COVID-19. Consistent with the literature, this research found 43.75% of mothers with BMI $\geq$ 30 kg/m2 (13).

Our study revealed maternal-fetal immunity, which occurs in two phases: before birth through maternal antibody transfer to the fetus, and after birth during lactation. The most significant finding of our study was the discovery of maternal-fetal immunity. This occurs in two stages: before birth through the transfer of antibodies from the mother to the fetus, and after birth during the lactation period (14). Our results show that 25% of maternal plasma samples tested positive for SARS-CoV-2-specific IgM and 75% for IgG. These results are similar to those of Fenizia et al., who found 32% IgM and 63% IgG in mothers' plasma (15). Additionally, two mothers tested negative for both IgG and IgM, indicating an early stage of infection. IgG crosses the placenta

passively during late pregnancy, while IgM cannot due to its macromolecular structure (16). We detected SARS-CoV-2-specific IgG in both placental and umbilical cord blood of two newborns, whose mothers had SARS-CoV-2-specific IgG. The newborns were not SARS-CoV-2 RNA positive, did not require ventilation, and did not require any specific medication. These findings align with other studies that suggest the presence of maternal IgG in cord blood and placenta (17). However, vaccination should also be considered. Jones et al. reported a case of a vaccinated mother with positive antibody against SARS-CoV-2 in umbilical cord blood (18). Additionally, some studies suggest that the absence of antibodies in cord blood may be due to timing of infection or decline in placental antibody concentration during the second or third trimester of pregnancy (17).

The analysis of serological data showed the presence of IgM in the breastmilk of one mother who tested positive for IgM and negative for IgG. Previous studies have suggested that breastmilk, with its components such as SIgA (90%) and SIgM (8-15%), IgG (2-5%), and cytokines, provides protection to newborns against infections for up to six months after birth. (19). Our results differ from some studies that detected IgG and SIgA in breastmilk (20), but is broadly consistent with the other studies (21). A possible explanation for these results may be the fact that milk IgG originates from serum and only 11.8% of SARS-CoV-2 IgG was detected in blood samples one week after infection, taking about three months to reach its peak (100%) (22). Further follow-up of this mother could help investigate the presence of other antibodies in her breastmilk. Our study, like most investigations, did not find SARS-CoV-2 RNA in mothers' milk. These data contradict limited studies claiming the possibility of SARS-CoV-2 transmission through breastmilk , although none of them attempted to culture the SARS-CoV-2 isolates of positive milks to assess their infectivity (23). Therefore, given the limited research on the breastmilk of COVID-infected mothers, it is recommended that mothers take hygienic precautions such as wearing facemasks, washing their hands, and disinfecting surfaces during the lactation period .

In this study, the authors aimed to investigate the connection between SARS-CoV-2 and gestational complications, building on previous research that warned of the viral family's detrimental effects on pregnancy outcomes such as preterm birth (PTB), fetal growth restriction (FGR), low birth weight (LBW), preterm labor, and stillbirth. The study found a PTB rate of 62.5%, a CS rate of 62.5%, a LBW rate of 50%, and a neonatal death rate of 18.75%. These rates are comparable to those reported in review articles, which showed varying rates of PTB (14.3% - 61.2%) and neonatal death (0-11.7%) and an increasing trend of CS and LBW in mothers with

COVID-19 (24). Pregnancy is associated with several physiological, immunological, and hormonal changes that make women more susceptible to respiratory infections, and the suppressed immune system common during pregnancy can contribute to the progression of infections. Placental hypoxia can result in cytokine storms, which can damage the placenta, cause fetal growth restriction, preterm birth, and even abortion. Moreover, inflammatory factors prompt endothelial dysfunction, a certification of PE, end-organ damage, fetal hypoxemia and finally fetal distress (25).

One potential limitation of the current study is the small sample size. Furthermore, it is regrettable that the research did not include women in their first and second trimesters of pregnancy. Additionally, the study did not investigate the potential damage of SARS-CoV-2 on the placenta, which could provide valuable data on the relationship between SARS-CoV-2 and pregnancy complications. Lastly, the lack of an appropriate ELISA kit precluded the study from examining SIgM and SIgA levels in breastmilk.

The primary goal of this study was to assess the possibility of vertical transmission of SARS-CoV-2 and its effects on pregnancy outcomes. Our findings did not show evidence of vertical transmission, but revealed that pregnant women infected with SARS-CoV-2 were at risk of developing preterm birth, low birth weight, and cesarean delivery. Despite the limited sample size, this study provides valuable insights into the impact of SARS-CoV-2 on pregnancy. Further research is needed to fully understand the mechanisms and effects of SARS-CoV-2 on pregnancy, as well as to develop strategies to prevent or reduce adverse maternal and neonatal outcomes.

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Authors' contributions

AT, and SDH conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript. AT, SDH, ZS, EK, PC, MY and MB designed the data collection instruments, coordinated and supervised data collection. SDH, MB, MY, MR, MH collected data. MB collected and reviewed the radiological images. AT, SDH, MH, PC, and MR carried out the initial analysis. All authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

Ethics

Our study involving pregnant women with laboratory-confirmed COVID-19 adhered to ethical guidelines. We ensured transparency and respect for participants' autonomy. We initiated the informed consent process, providing clear and comprehensible information, and detailed written consent forms that outlined the study's purpose, procedures, and confidentiality. Patients were given ample time to review and ask questions, emphasizing the voluntary nature of their involvement. This ethical framework was maintained throughout the study period, with ongoing communication channels to address any inquiries or concerns. Our goal was to protect the rights and well-being of the pregnant women involved in our research.

Conflict of Interest

The authors declare no conflict of interest in this study

Funding statement

Ethical approval for data and specimen collection was granted by the Ethics Committee of Golestan University of Medical Sciences (Ethics code: IR.Goums.REC.1399.176).

Data availability

All data generated or analyzed during this study are included in this article and its supplementary material files. Further enquiries can be directed to the corresponding author.

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Figure legends

Table 1: The clinical and laboratory characteristics, and treatments of pregnant women

Table 2: Maternal SARS-CoV-2 serological and molecular outcomes

Table 3: Neonatal outcomes

Figure 1: Chest CT scan (transverse plane) of seven patients.