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A critical assessment of the potential vertical transmission hypotheses: Implications for research on the early-life infection with COVID-19

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ARTICLE INFO

Keywords: COVID-19 Systematic review Neonate Transmission routes Preventative proposals

ABSTRACT

The risk of potential vertical transmission in SARS-CoV-2 infected pregnant women is currently a topic of debate. To explore the correlation between the two, we searched PubMed, Embase®, and Web of Science for studies on vertical transmission of COVID-19. The quality of the studies was evaluated by the Cochrane risk of bias tool. Detailed information of each included case including methods of delivery, protection measures for mothers and neonates at birth, types of specimens, inspection time, results of testing and feeding patterns was collected to assess the possibility of vertical transmission. The results showed that of the 390 neonates reported in 36 studies, 23 were infected with SARS-CoV-2 by potential vertical transmission. From the perspective of virology and pathology, vertical transmission of SARS-CoV-2 was possible via uterus or breastmilk. Some reported potential vertically transmitted neonates could be attributed to horizontal transmission. It is extremely vital to fully elucidate the potential routes of transmission of SARS-CoV-2, implicating clinical practice and nursing to reduce the risk of not only horizontal transmission but also vertical transmission, thus protecting neonates from COVID-19 infection.

1. Introduction

The coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has spread rapidly across the world. Based on current evidence, COVID-19 is mainly transmitted by close contact through respiratory droplets [1]. Until now, vertical transmission route of COVID-19 has not been confirmed. Vertical transmission is defined as passage of pathogens from mother to an embryo, fetus, or baby mainly across the placenta, through the female reproductive tract or in the breast milk during the period immediately before and after birth [2].

It has been documented that pregnant women are more susceptible to some infectious diseases, including respiratory pathogens, which hints a higher risk of COVID-19 infection [3,4]. The risk of potential vertical transmission in COVID-19 pregnant patients is currently a topic of debate. Early series reports show no SARS-CoV-2 detected in the amniotic fluid, vaginal mucus, cord blood, or neonatal throat swabs immediately after birth [5,6]. SARS-CoV-2 strains show 50% and 79% sequence’ homology to previous pandemics SARS-CoV and MERS-CoV respectively [7]; there are no vertically transmitted cases of SARS (severe acute respiratory syndrome) and MERS (middle east respiratory syndrome) [8–10], standing for non-existent vertical transmission. However, a few neonatal series and case reports indicate very early infections in infants born to SARS-CoV-2 positive mothers [11,12], which raises concerns on vertical transmission of SARS-CoV-2.

The eventual vertical transmission route is still not fully understood. In this systematic review, discussions were carried out on the possible routes of vertical transmission and horizontal transmission. The importance of vertical transmission on the future health of newborns was also discussed. The objective was to better understand the potential vertical transmission and implications for neonatal health outcome.
2. Methods

2.1. Data sources and searches

A systematic electronic search was carried out in PubMed, Embase® and Web of Science by two authors independently. The keywords and Mesh terms were listed as ‘COVID-19’ OR ‘SARS-CoV-2’ OR ‘coronavirus’ AND ‘maternal neonatal’ OR ‘maternal fetal’ OR ‘vertical transmission’ OR ‘intrauterine transmission’ OR ‘mother child transmission’. The Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines were followed with no language restrictions.

2.2. Study selection

All relevant papers published up to November 30, 2020 were initially screened according to titles and abstracts. Reduplicative records and studies not relevant to present work were excluded. Secondary data and reviews were excluded. Then the full texts of remained papers covering case reports, cross section studies and prospective cohort studies were obtained and read carefully for final check. Overall, 36 studies were included in the final analysis (Fig. 1).

2.3. Quality assessment and data extraction

The Cochrane risk of bias tool was applied for study quality
assessment [13]. For each study, detailed information including methods of delivery, protection measures for mothers and neonates at birth, types of specimens, inspection time, results of testing and feeding patterns was extracted.

3. Results

3.1. Summary

After having reviewed the available literature, most of evidence supported that mother-to-child SARS-CoV-2 transmission did not occur and merely 23 out of 390 neonates from all over the world were suspected vertically transmitted (Table 1). We focused on the 23 cases, considering the positive rate (5.897%) was rather low. The detailed information of characteristics was listed as follows (Table 2). We sought to illustrate the credibility and the potential defect of each suspected case, which can help support viewpoint of vertical transmission more clearly and strongly. In addition, the risks of bias in the 36 included studies were variable. Few had a low risk of bias in every domain. The results are summarized in Table 1.

Table 1

| Studies | Sources | Neutones, total (cases) | Risk of bias |
|---------|---------|------------------------|--------------|
|         |         | 390 (23) | Reporting Bias | Information Reporting Bias | Diagnostic Bias |
|         |         |           | + | – | – |
| Vivanti AJ et al. [14] | Antoine Bécèrè Hospital, Paris, France | 1 (1) | + | – | – |
| Dong, L. et al. [5] | Renmin Hospital, Wuhan, China | 1 (1) | + | – | + |
| Zhang, Z.J. et al. [21] | Renmin Hospital, Wuhan, China | 4 (4) | + | – | – |
| Zeng, L. et al. [12] | Wuhan Children’s Hospital, Wuhan, China | 33 (3) | – | – | – |
| Yu, N. et al. [11] | Tongji Hospital, Wuhan, China | 3 (1) | – | + | – |
| Wang, S. et al. [24] | Tongji Hospital, Wuhan, China | 1 (1) | + | – | – |
| Hu, X. et al. [25] | Tongji Hospital, Wuhan, China | 7 (1) | – | – | – |
| Sun, M. et al. [20] | People’s Hospital, Zhengzhou, China | 3 (2) | – | + | + |
| Zamaniany, M. et al. [15] | Imam Khomeini Hospital, Sari, Iran | 1 (1) | + | – | – |
| Alzamora, M.C. et al. [23] | British American Hospital, Peru | 1 (1) | + | – | – |
| Patane, L. et al. [16] | Papa Giovanni XXIII Hospital, Italy | 22 (2) | – | + | – |
| Ferrazzi, E. et al. [22] | 12 hospitals, Italy | 42 (3) | – | + | – |
| Groí, R. et al. [19] | Ulm University Medical Center, Germany. | 2 (2) | + | – | – |
| Chen, H. et al. [15] | Zhongnan Hospital, Wuhan, China | 6 (0) | – | – | – |
| Xiong, X. et al. [20] | YouAn Hospital & Capital Medical University, China | 1 (0) | – | – | – |
| Yang, P. et al. [40] | Zhongnan Hospital, Wuhan, China | 6 (0) | – | + | – |
| Peng, Z. et al. [41] | Chongqing Three Gorges Central Hospital, China | 1 (0) | – | – | – |
| Liu, W. et al. [42] | Tongji Hospital, China | 19 (0) | + | – | – |
| Chen, R. et al. [43] | Renmin hospital, Wuhan, China | 17 (0) | – | – | – |
| Fan, C. et al. [44] | Renmin Hospital, Wuhan, China | 2 (0) | – | + | – |
| Khan, S. et al. [45] | Renmin Hospital, Wuhan, China | 3 (0) | – | + | – |
| Wu, Y. et al. [46] | Renmin Hospital, Wuhan, China | 5 (0) | – | – | – |
| Cao, D. et al. [47] | Maternal and Child Health Hospital of Hubei Province, China | 11 (0) | – | – | – |
| Li, N. et al. [48] | Hubei Provincial Maternal and Child Health Center, China | 16 (0) | – | – | – |
| Lu, D. et al. [49] | The No. 2 Affiliated People’s Hospital, Anhui Medical University, China | 1 (0) | – | – | – |
| Li, Y. et al. [50] | The First Affiliated Hospital, Zhejiang University, China | 1 (0) | – | – | – |
| Chen, S. et al. [51] | Union Hospital, Wuhan, China | 3 (0) | – | + | – |
| Zhang, L. et al. [52] | Renmin Hospital of Wuhan University & The Central Hospital of Qianjiang City, China | 10 (0) | – | – | – |
| Zhu, H. et al. [53] | 5 hospitals, Hubei, China | 9 (0) | – | – | – |
| Chen, Y. et al. [54] | Hospitals in Wuhan, China | 3 (0) | – | + | – |
| Qiancheng, X. et al. [55] | Hospitals in China | 23 (0) | – | + | – |
| Yan, J. et al. [56] | 25 hospitals in China | 86 (0) | – | + | – |
| Blauvelt, C.A. et al. [57] | San Francisco, California, the USA | 1 (0) | – | – | – |
| Breslin, N. et al. [58] | New York City hospitals, the USA | 43 (0) | – | – | – |
| Lowe, B. and B. Bopp [59] | Gold Coast University Hospital, Australia | 1 (0) | – | – | – |
| Lyra, J. et al. [60] | a level III hospital in Porto, Portugal | 1 (0) | – | + | – |

Noted.

Plus means high risk of bias while minus means low risk of bias.

Reporting bias: whether non + infected neonates were reported.

−: SARS-CoV-2 negative neonates reported, with negative neonates.

+: Only SARS-CoV-2 positive neonates reported.

Information reporting bias: whether related information (methods of delivery, etc.) was described.

−: For the case with the most missing items, the number of missing items ≤ 1.

+: For the case with the most missing items, the number of missing items ≥ 2.

Diagnostic bias: whether the diagnosis of SARS-CoV-2 infection is supported by nucleic acid test.

−: Cases were diagnosed only based on positive nucleic acid test both in mothers and neonates.

+: Rather than positive nucleic acid test, cases were diagnosed based on other diagnostic methods, such as CT scan, etc. both in mothers and neonates.
### Table 2
Detailed information 23 cases with potential vertical transmission.

| Features | Vivanti AJ et al. [14] | Dong, L. et al. [6] | Zhang, Z.J. et al. [21] | Zeng, L. et al. [12] | Yu, N. et al. [11] | Hu, X. et al. [25] |
|----------|---------------------------|----------------------|------------------------|---------------------|-----------------|------------------|
| Case 1   | Case 2                     | Case 3                | Case 4                 | Case 5              | Case 6          | Case 7           |
| Mothers' status | NICU                      | NICU                  | Non-ICU                | Non-ICU             | Non-ICU         | Non-ICU          |
| Infants' status | NICU                      | Non-NICU              | NICU                   | Non-NICU            | Non-NICU        | Non-NICU         |
| Delivery | Cesarean section          | Cesarean section      | Cesarean section       | Cesarean section    | Cesarean section| Cesarean section |
| Protection for mothers | Yes                      | Yes                   | Yes                    | Yes                 | Yes             | Yes              |
| Protection for infants | Yes                      | No                    | Yes                    | Yes                 | Yes             | Yes              |
| Infants' positive rate (positive/all) | 1/1                      | 1/1                   | 4/4                    | 3/33                | 1/3             | 1/7              |
| Infants' COVID symptoms and time | Shortness of Breath; the same day as the nucleic acid diagnosis | Fever, cough, vomiting; 2 days between symptom and nucleic acid diagnosis | No | Fever; the same day as the nucleic acid diagnosis | No | No |
| Infants' specimens | Amniotic fluid, blood, placenta(+) | Blood, NP swabs | NP swabs | swabs | NP swabs | Throat swabs(+) |
| | Nucleic acid test (+) | Nucleic acid test (+), cytokine test (+) | Nucleic acid test (+), CT scan (+) | Nucleic acid test (+), CT scan (+) | Nucleic acid test (+), CT scan (+) | Nucleic acid test (+) |
| | Inspect time | 2 h after birth | PND 2 | PND 3 | PND 5 | PND 2 |
| | Breastfeeding | No | Yes | No | Yes | No |

| Features | Wang, S. et al. [24] | Sun, M. et al. [20] | Zamaniyan, M. et al. [13] | Alzamora, M. C. et al. [23] | Patané, L. et al. [16] | Ferrazzi, E. et al. [22] | Groiß, R. et al. [19] |
|----------|-----------------------|---------------------|---------------------------|---------------------------|------------------------|------------------------|----------------------|
| Case 12  | Case 13                | Case 14             | Case 15                    | Case 16                   | Case 17                | Case 18                | Case 19              |
| Mothers' status | Non-ICU               | Cesarean section | NICU | Cesarean section | NICU | Cesarean section | NICU | Cesarean section |
| Infants' status | CESAREAN SECTION | NICU | Cesarean section | NICU | Cesarean section | NICU | Cesarean section |
| Delivery | Cesarean section      | Cesarean section    | NICU | Cesarean section | NICU | Cesarean section | NICU | Cesarean section |
| Protection for mothers | Yes                   | Yes                 | Yes | Yes                 | Yes | Yes             |
| Protection for infants | Yes                   | No                  | Yes | Yes                 | Yes | Yes             |
| Infants' positive rate (positive/all) | 1/1                   | 2/3                 | 1/1 | 2/22                | 3/42 | 2/2 |
| Infants' COVID symptoms and time | Fever; PND 3 | Fever; PND 0 | mild respiratory difficulty, cough; PND 6 | respiratory symptoms; PND 3 | respiratory symptoms; PND 7 | breathing problems; PND 8 |
| Infants' specimens | Pharyngeal swabs, milk samples(−) | Pharyngeal, laryngeal, throat, tracheal tube tip swabs | Pharyngeal, laryngeal, throat, tracheal tube tip swabs | Amniotic fluid(+) | cord blood(−), nasal and throat swabs(−) | NP swabs | NP swabs, placenta (+) | Throat swabs |
| | | | | | NP swabs, placenta (+) | Throat swabs | Throat swabs | Throat swabs |
| | | | | | | | OP and NP swabs(−), milk samples(−) | OP and NP swabs(−), milk samples(−) |

(continued on next page)
the transplacental transmission of SARS-CoV-2 in an infected neonate delivered by cesarean section and presenting with neurological compromise. RT-PCR (Reverse Transcription-Polymerase Chain Reaction) on the placenta was positive for both SARS-CoV-2 genes and viral load was much higher in placental tissue than in amniotic fluid and maternal or neonatal blood, which validated the existence of SARS-CoV-2 transplacental transmission from the perspective of virology and pathology. Besides, according to a case report in Imam Khomeini Hospital, Sari, Iran [15], the neonate who was delivered by cesarean section developed fever on birth, amniotic fluid sample was tested positive, hinting intrauterine infection. There were two infected neonates vaginally delivered in Papa Giovanni XXIII Hospital Bergamo-Italy [16], the two neonates did not show any symptoms related to COVID-19, and the direct visualization of SARS-CoV-2 RNA in the infected placenta rang the alarm bell of the possible intrauterine infection; the SARS-CoV-2 receptor ACE2 expression of maternal-fetal interface also confirmed the risk of infection in utero [17]. Additionally, the elevated IgM antibody level in neonatal blood sample suggested that the neonate was infected in utero [18], in other word, transmitted transplacentally. Of note, researchers from Germany detected SARS-CoV-2 RNA in milk samples from mother [19], indicating SARS-CoV-2 could be vertically transmitted via breast milk.

### 3.3. Evidence lack of credibility

Although there were more COVID-19 neonatal cases reported transmitted vertically, some leaks or defects were obtained and several unaddressed issues were presented. 1) **Contact infection or nosocomial infection** Regarding some cases, strict infection control and prevention procedures were conducted, however, in other cases, chance of nosocomial infection could not be ruled out. As for those infected babies discharged home [20–22], it was more ambiguous that the virus was spread via airborne droplets, personal contact or in vertical ways. 2) **Types of samples for detection** In many cases above, only one or two maternal and neonatal samples were tested [6,11,20], few RT-PCR testing of amniotic fluid or placenta was performed, not corresponding to golden standard for clinical diagnosis. 3) **Sterile operation procedures for samples** Sterile operation procedures for extracting and transferring samples should have been carried out; yet few studies mentioned it to avoid contamination. 4) **Delay for SARS-CoV-2 detection** Plenty of studies [23] in which researchers claimed the possible vertical transmission had the common limitation, not testing for the virus immediately after birth, increasing the risk of contamination [11,12,24,25]. It was important to emphasize that maternal and neonatal samples needed to be collected and tested immediately after delivery, thus guaranteeing the samples were not contaminated and best represented the true condition. 5) **Lack of longitudinal follow-up for infants** It could be more convincing to conduct longitudinal follow-up for suspected infants with COVID-19 infection, distinguished from other virus infection. And the testing results might be false positive or false negative, which meant just one testing could not draw a valid conclusion.

### 3.4. Potential routes of transmission

It has been well-known that droplet transmission and contact transmission are main routes of COVID-19 transmission. The possibility of vertical transmission is still controversial. The potential routes of transmission were summed up and visualized in Fig. 2. Based on synthetic analysis of all suspected vertically transmitted neonates with COVID-19, the potential routes of vertical transmission were summarized as follows: 1) **Intrauterine or transplacental infection** The elevated IgM antibody level [96.2% specificity of IgM for SARS-CoV-2] in neonatal blood sample suggested that the neonate was infected in utero [6]. The SARS-CoV-2 receptor ACE2 expression of maternal-fetal interface also stood for the possible intrauterine infection [17]. 2) **Infection...
through the female reproductive tract While the transmission of SARS-CoV-2 through female reproductive tract has not yet been established, the obstetricians preferred to perform caesarean section to reduce the risk of vertical transmission at delivery [26]. Furthermore, it is very important to shorten time of caesarean section to minimize the contact to maternal blood and body fluid, given SARS coronavirus was detected in the maternal peritoneal fluid collected during cesarean section in 28 SARS patients [27].

3) Breastfeeding infection SARS-CoV-2 was detected in breastmilk [19], indicating breastfeeding infection might exist. Based on potential transmission routes, infants’ placenta, amniotic fluid, blood, breast milk, oral, nasal and nasopharyngeal samples need to be detected. However, a case report from Mexico [28] showed that on the fourth day after delivery, real-time RT-PCR analyses of the mother’s milk and stool samples were positive for SARS-CoV-2 RNA, a similar result was obtained for the infant’s stool sample. What’s more, according to a retrospective cohort study in Zhejiang, China [29], viral RNA was detected in the stool of 59% (55/93) of patients. The median duration of viral RNA in stool was 22 days. The duration of SARS-CoV-2 infection is significantly longer in stool samples than in respiratory and serum samples. Therefore, it is important to highlight the need to strengthen the management of infants’ stool samples in the prevention and control of the epidemic.

3.5. Proposals on clinical practices

Vertical transmission was presented in few studies, because this was a rare occurrence using the methods and samples sets studied. Therefore, it is extremely vital to avoid not only horizontal transmission such as droplet infection and contact infection, but also potential vertical transmission including intrauterine infection, infection through the female reproductive tract and breastfeeding infection (Fig. 3 a), appropriate isolation and postpartum care should be done at birth and after birth, thus protecting neonates from COVID-19 infection.

Moreover, to confirm vertical transmission of SARS-CoV-2 more accurately and precisely, strict infection control and prevention procedures should be conducted to prevent contact infection or nosocomial infection such as mothers’ wearing N95 masks, all physicians’ wearing N95 mask, glasses and gloves and prohibition of close contact, more samples including amniotic fluid, cord blood, neonatal throat swabs, and breastmilk ought to be collected and detected immediately after birth with sterile operation procedures and longitudinal follow-up should be implemented (Fig. 3 b).

4. Discussion

The type of pneumonia caused by the 2019 novel coronavirus disease (COVID-19) is a highly infectious disease, and the ongoing outbreak has been declared by WHO as a global public health emergency [30]. At the same time, COVID-19 will have great effects on maternal, perinatal and neonatal outcomes [31]. It is important to know whether vertical transmission exists and its possible transmission routes for the long-term healthy development of infants and young children, the promotion of community health and disease prevention.

After having reviewed the available literature and arguing about vertical transmission as well as horizontal transmission, we assessed 23 suspected vertically transmitted neonates and summarized the valid evidence as well as the limitations and defects of included studies. Subsequently, we gave recommendations on clinical practices to pursue the best accuracy and precision for COVID-19 diagnosis.

The most convincing evidence of vertical transmission would be to confirm the replication of SARS-CoV-2 in maternal and neonatal samples including placenta, amniotic fluid, cord blood and pharyngeal swabs.
collected immediately after birth, using aseptic technique. Strict protection and prevention for mothers and neonates ought to be taken to exclude the possibility of contact infection or nosocomial infection. What’s more, having a COVID jab is necessary for mothers and neonate, because it is effective in preventing disease development and reducing deaths [32]. Future studies should specify if patients have had the immunization, which one and when. Additionally, longitudinal follow-up for suspected infants is required. Cumulative information of mothers as well as infants with COVID-19 was necessary to better knowledge the effect of SARS-CoV-2 on maternal and fetal health.

Most of the previous studies supported for vertical transmission being not existing, nevertheless this chance could not be completely ruled out [33–38]. On the basis of our study, we draw a conclusion that vertical transmission of SARS-CoV-2 was possible via intrauterine transmission or breastfeeding. However, other cases of vertical transmission in our study could not be completely persuasive due to the leaks and defects. As a result of the newborns’ problem of transferring from one hospital to another, contact infection or nosocomial infection could not be excluded in some cases [11,24]. There were limited maternal and neonatal samples being tested and delay for SARS-CoV-2 detection was possible [38]. On the basis of our study, we draw a conclusion that vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records, Lancet 395 (10226) (2020) 809–815.

Despite a novel method based on delineating the credibility and defect of each case was employed to explore this fascinating topic, there were some limitations of the study. Firstly, statistical inference was not performed on account of limited amount of positive cases with too many confounding factors. Although most studies have used strict quarantine measures to rule out other routes of transmission, merely 23 out of 390 neonates included from all over the world were suspected vertically transmitted. Secondly, although we have put effort on removing duplicates according to characteristics of each case, there still could be possible duplicated cases. Thirdly, more researches should pay attention to potential intrauterine transmission mechanistically to figure the possibility out. Last, due to publication bias, researchers tended to report positive cases.

We sought to contribute to the discussion of the contested topic and get a better understanding of vertical transmission for COVID-19, thus promoting maternal and fetal health. Based on this assessment, scientific evidence validated that SARS-CoV-2 vertical transmission could occur via uterus or breastfeeding and we discussed the implications for clinical practices and future researches.

Declaration of competing interest

Mengqin Yang, Qiuqin Wang, Yulei Song, Minyan Zou, Yan Li, Guihua Xu, Ting Yan and Yamei Bai declared that they have no conflict of interest.

Acknowledgements

Mengqin Yang and Qiuqin Wang contributed to literature search, data collection and drafted the manuscript. They contributed equally to the work.

Yulei Song, Minyan Zou, Yan Li, Guihua Xu and Ting Yan contributed to literature search and carefully revised the manuscript.

Yamei Bai designed the study and carefully revised the manuscript.

All authors read and approved the final manuscript.

Abbreviations

COVID-19 severe acute respiratory syndrome coronavirus 2
PRISMA Preferred Reporting Items for Systematic Review and Meta-Analyses
RT-PCR Reverse Transcription-Polymerase Chain Reaction

Funding

This work was supported by the National Natural Science Foundation of China [grant numbers 72004102];

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