RESEARCH ARTICLE

# Investigating vertical transmission and maternofoetal outcomes in pregnant women with COVID-19: a case-control study from Pakistan

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## Abstract

**Objective:** To estimate the frequency of severe acute respiratory syndrome coronavirus-2 among pregnant women, the impact in terms of obstetrical and clinical outcomes and vertical transmission to the neonates.

**Method:** The prospective, case-control study was conducted at Zainab Panjwani Memorial Hospital, Karachi, from March to December 2021, and comprised pregnant women regardless of gestational age who exhibited symptoms or had a suspicion of exposure to any confirmed coronavirus disease-2019 individual. They were screened for severe acute respiratory syndrome coronavirus-2 infection using polymerase chain reaction or serology. Those who tested negative were designated as control group A, while who had a positive serology result along with a negative polymerase chain reaction were taken as recovered case group B1, and those who tested positive for polymerase chain reaction were called the positive case group B2. Groups B1 and B2 were followed up till delivery. The clinical presentation of coronavirus disease-2019 infection in pregnancy and its obstetrical and neonatal outcomes was assessed. Products of conception were tested for the detection of the severe acute respiratory syndrome coronavirus-2 genome. The viral genome from group B2 cases was sequenced to confirm vertical transmission. Data was analysed using GraphPad Prism V8.

**Results:** Of the 139 pregnant women, 74(53.2%) were in group A with mean age 25.87±6.90 years, 49(35.3%) were in group B1 with mean age 25.53±7.02 years, and 16(11.5%) were in group B2 with mean age 27.12±5.03 years. The gestational age at which termination of pregnancy occurred was 38.3±1.26 weeks in group B1 and 38.3±1.85 weeks for group B2. There were 96 neonates across the 3 groups. Of the 11(11.45%) neonates in group B2, 1(9.09%) had postnatal transmission of severe acute respiratory syndrome coronavirus-2 and this mother-neonate case was taken as the Indexed case. The severe acute respiratory syndrome coronavirus-2 genome isolated from the neonate showed similar mutations as the viral strain infecting the mother.

**Conclusion:** The risk of vertical transmission was found to be low. The severe acute respiratory syndrome coronavirus-2 genome was the same for both the mother and the neonate.

**Key Words:** SARS-CoV-2, vertical transmission, pregnancy, COVID-19, Pakistan

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## Introduction

Pregnant women and their foetuses/neonates are regarded as the vulnerable group against coronavirus disease-2019 (COVID-19) infection. Since early 2020, conflicting evidence on vertical transmission of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) virus and adverse maternal outcomes have been reported globally[1-3]. Moreover, pregnant populations belonging to low- and middle-income countries (LMICs) have been reported to have higher miscarriages and stillbirths as well as neonatal SARS-CoV-2 infections[4]. One case study from Pakistan reported a first-trimester miscarriage, while another reported a possible in-utero infection[5,6]. Other studies reported good outcomes for both mother and the foetus during the third trimester in COVID-19 infection cases[7,8]. To our knowledge, no conclusive and detailed investigative study has been done in Pakistan in this regard with a significant sample size. The current study was planned to fill the gap by estimating the frequency of SARS-CoV-2 vertical transmission in neonates born to pregnant women, and to evaluate the impact of SARS-CoV-2 infection during gestation in terms of obstetrical and clinical outcomes for both mothers and their neonates.

## Subjects and Methods

The case-control study was conducted at the Zainab Panjwani Memorial Hospital, Karachi, from March to

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December 2021. Approval was obtained from institutional ethics review committee as well as from the National Bioethics Committee of Pakistan. The sample size was calculated using OpenEpi[9] with confidence limit 0.05, and frequency of vertical transmission 3.2%[10]. The obtained sample size was good enough for achieving 95% confidence interval (CI).

The sample size was raised using consecutive sampling technique. Those included were pregnant women regardless of gestational age who were exhibiting symptoms or had a suspicion of exposure to any confirmed COVID-19 case. They were screened for SARS-CoV-2 infection after obtaining informed consent from each of them. At the registration (T0) stage, a nasopharyngeal swab and a blood sample were obtained for reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) and lateral immunoassay for antibody detection, respectively. A data-collection questionnaire was also filled out at the time of registration (T0 stage).

Those who tested negative were designated as control group A, while those who had a positive serology result along with a negative RT-qPCR result were taken as recovered case group B1, and those who tested positive on RT-qPCR were called the positive case group B2. Groups B1 and B2 were followed up till delivery (T2 stage).

At T2 stage, in addition to maternal and neonatal nasopharyngeal swabs and serum, products of conception were also collected, including placental and umbilical cord tissue, cord blood and amniotic fluid. A second questionnaire regarding obstetrical and neonatal outcomes was also completed. Follow-up (T3 stage) of these mother-neonate pairs was planned at 48 hours post-birth and then a week later. The neonate's activity was recorded with an appearance-pulse-grimace-activity-and-respiration (APGAR) score at 1 minute[11]. On follow-ups, only maternal and neonatal swabs and blood samples were collected. Breastmilk was also collected at 48-hour follow-up.

Data related to control group A women was added from the questionnaires filled at the T0 stage and from their medical records.

In the case of such subjects, from group B2, that had an active SARS-CoV-2 infection subjects at the T2 stage, nasopharyngeal swab specimens of all the staff, including doctors and paramedics, who assisted these births, and the attendants accompanying these subjects were also collected. Similarly, the beddings that were in use by the patients and the air-conditioning (AC) vents of the designated isolation wards were also swabbed for the virus.

Viral Transport Media (VTM) (Yaohja Medical, China) was used to collect and store the nasopharyngeal swabs. Peripheral and cord blood samples were spun for 10 minutes at 3000x. Serum was then used for rapid antibody detection on Quick Profile COVID-19 immunoglonulin G IgG/IgM Test Card (Lumiquick Diagnostics, United States). Amniotic fluid and breastmilk were collected in 15ml falcon tubes containing VTM under sterilised conditions. Breastmilk samples were processed by centrifuging thrice at 14,000rpm for 5 minutes. The supernatant layer was then used for viral ribonucleic acid (RNA) extraction. Placenta and cord tissues were collected in vials filled with DNA/RNA shield (ZymoResearch, USA) and crushed in liquid nitrogen before extraction. Viral RNA was isolated from the samples using QIAamp viral RNA mini kit (Qiagen, USA). The extracted RNA was amplified to detect SARS-CoV-2 using Bosphore Novel Coronavirus Detection Kit v4 (Anatolia Diagnostics, Turkey) on ABI7500 Fast PCR System (Applied Biosystems, USA). Sample processing, viral RNA extraction, and detection were performed at the Biosafety level III (BSL-III) laboratory at the National Institute of Virology, International Centre for Chemical and Biological Sciences (ICCBS), University of Karachi.

The isolated viral RNA was reverse-transcribed to double-stranded complementary DNA using the Maxima H Minus Double-Stranded complementary DNA (cDNA) Synthesis Kit (ThermoFisher Scientific, USA). Following the kit's instructions, the reverse-transcribed double-stranded cDNA was used for library preparation using the Illumina DNA Prep with Enrichment kit (Illumina Inc., USA). The library was subjected to paired-end sequencing on the Illumina MiSeq platform (Illumina Inc., USA).

The generated raw sequence reads in the form of fastq files were assembled into the contigs, to produce continuous representation of SARS-CoV-2 genome, using a web-based Genome Detective virus tool (V1.133)[12]. For variant detection, the DNA reads were mapped against the SARS-CoV-2 reference genome (NC_045512.2) as per the MiSeq on-instrument variants detection pipeline[13]. The lineages of SARS-CoV-2 genomes were determined according to the Phylogenetic Assignment of Named Global Outbreak Lineages (PANGOLIN 2.0) software suite[14].

Data was analysed using GraphPad Prism V8. Frequencies and percentages were used to express qualitative characteristics, while mean +/- standard deviation values were used to report quantitative variables.

## Results

Of the 139 pregnant women, 74(53.2%) were in group A with mean age 25.87±6.90 years, 49(35.3%) were in group B1 with mean age 25.53±7.02 years, and 16(11.5%) were in group B2 with mean age 27.12±5.03 years. The baseline characteristics of the groups at the T0 stage were noted in detail (Table 1).

None of the recruited subjects had received COVID-19 vaccination during their pregnancy. The most common symptoms were fever and cough (Figure 1). No subject in the cohort developed pneumonia.

**Table-1:** Characteristics of the participants at baseline (T0).

| Characteristics | Group A (n= 74) | Group B1 (n=49) | Group B2 (n=16) |
|---|---|---|---|
| Maternal age, years, Mean± SD | 25.87±6.90 | 25.53±7.02 | 27.12±5.03 |
| Gestational age, weeks, Mean± SD | 34.44±5.58 | 35.83±4.97 | 32.87±7.5 |
| Symptomatic, n (%) | 32 (43.24) | 17 (34.6) | 12 (75) |
| Asymptomatic, n (%) | N/A | 32 (65.30) | 4 (25) |
| Primigravida, n (%) | 27 (36.4) | 22 (44.8) | 6 (37.5) |
| Multigravida, n (%) | 46 (62.16) | 26 (53.06) | 8 (5) |
| Unknown, n (%) | 1 (1.35) | 1 (2.04) | 2 (12.5) |
| Received COVID-19 vaccine, n (%) | 0 | 0 | 0 |
| Travel history in last 14 days, n (%) | 0 | 0 | 0 |
| Confirmed contact with an infected person, n (%) | 1 (1.35) | 1 (2.04) | 0 |

COVID-19: Coronavirus disease 2019, SD: Standard deviation.

From group B1, 37(75.51%) subjects, and from group B2, 11(68.75%) subjects were followed up till the T2 stage. For group A, complete data was available for 47(63.5%) cases. The gestational age at which termination of pregnancy occurred was 38.3±1.26 weeks in group B1 and 38.3±1.85 weeks for group B2. There were 96 neonates born to 95(100%) mothers across the 3 groups, with 1 set of twins in group B1. All outcomes related to the mothers and the neonates across the sample were noted in detail (Table 2). No neonatal death was observed. There were 21(44.68%) group A women who underwent caesarean (C) sections compared to 9(24.32%) in B1 and 3(27.2%) in B2 groups. The high number of C-sections in group A was because of several obstetrical reasons.

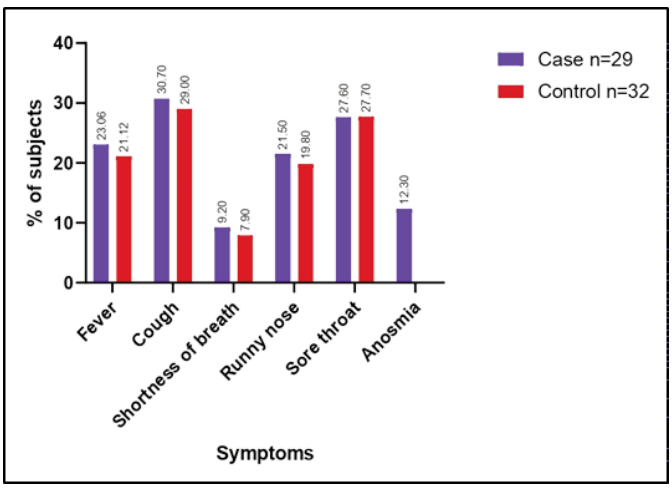


**Figure-1:** Common symptoms reported by controls (n=32) and cases (n=29).

**Table-2:** Obstetrical and neonatal outcomes at T2 stage.

| Outcomes | Group A (n= 74) | Group B1 (n=37) | Group B2 (n=11) |
|---|---|---|---|
| Termination of gestational age, weeks, Mean± SD | 37.7±1.40 | 38.3±1.26 | 38.3±1.85 |
| Preterm births, n (%) | 3 (6.4) | 2 (5.4) | 1 (9.09) |
| Neonates born, n | 47 | 38 (1 set of twins) | 11 |
| **Foetal Gender** | | | |
| Male, n (%) | 25 (53.1) | 24 (51.06) | 8 (72.72) |
| Female, n (%) | 20 (42.5) | 14 (37.8) | 3 (27.27) |
| Unknown, n (%) | 2 (4.25) | 0 | 0 |
| **Type of delivery** | | | |
| Vaginal, n (%) | 26 (55.31) | 29 (78.37) | 8 (72.72) |
| C- Sections, n (%) | 21 (44.68) | 9 (24.32) | 3 (27.2) |
| Emergency C-section, n (%) | 12 (25.5) | 4 (10.8) | 1 (9.0) |
| Elective C-section, n (%) | 9 (19.1) | 5 (13.5) | 2 (18.1) |
| Birth weight (kg), | 2.91±0.32 | 2.78±0.42 | 2.94±0.45 |
| **Mean± SD** | | | |
| Breastfeeding, n (%) | 33 (70.2) | 30 (78.9) | 7 (63.6) |
| Admission to NICU, n (%) | 30 (63.8) | 16 (42.1) | 3 (27.27) |
| Infected neonate, n (%) | N/A | 0 | 1 (9.09) |
| Neonatal death, n (%) | 0 | 0 | 0 |

PCR: Polymerase chain reaction, NICU: Neonatal intensive care unit, SD: Standard deviation.

All group B2 subjects had a mild to moderate course of the disease. To assess vertical transmission, all products of conception were tested for the SARS-CoV-2 genome and were found to be negative. Of the 11(11.45%) neonates in group B2, 1(9.09%) had postnatal transmission of SARS-CoV-2 (Subject No. 11) and this mother-neonate case was taken as the Indexed case.

The indexed woman was in her late 30s, with a complaint of severe coughing for a week. The patient was afebrile with a body temperature of 98°F while other vitals were also in the normal range (blood pressure 100/60, pulse rate 90 beats/min, respiratory rate 16 breaths/min). The patient was diagnosed with preterm premature rupture of membrane (PPROM) at 34 weeks of gestation. On the same day, her nasopharyngeal sample tested positive for SARS-CoV-2. The subject gave birth spontaneously to a premature baby weighing 2.1kg the following day. The neonate's APGAR score at 1 minute was 7. Breastfeeding was allowed, with the mother wearing a mask and

**Table-3:** Genetic mutations observed in the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) strains isolated from the index mother and her baby.

| Mutation | Amino acid change | Gene | Subject No. 11 (Mother) | Baby |
|---|---|---|---|---|
| **241C>T** | upstream | nsp1, nsp2 | HOM | HOM |
| **913C>T** | S36S | nsp2 | HOM | HOM |
| **4475C>T** | R586C | nsp3 | HOM | HOM |
| **5388C>A** | A890D | nsp3 | HOM | HOM |
| **5986C>T** | F1089F | nsp3 | HOM | HOM |
| **6954T>C** | I1412T | nsp3 | HOM | HOM |
| **8578G>A** | K8K | nsp4 | HET | HOM |
| **11287GTCTGGTTTT>G** | 106_108del | nsp6 | HOM | HOM |
| **14676C>T** | P412P | nsp12 | HOM | HOM |
| **15279C>T** | H613H | nsp12 | HOM | HOM |
| **16176T>C** | T912T | nsp12 | HOM | HOM |
| **21761G>T** | A67S | S | HOM | HOM |
| **21764ATACATG>A** | 68_70del | S | HOM | HOM |
| **21855C>T** | S98F | S | HET | HOM |
| **23063A>T** | N501Y | S | HOM | HOM |
| **23271C>A** | A570D | S | HET | HOM |
| **23403A>G** | D614G | S | HOM | HOM |
| **23604C>A** | P681H | S | HET | HOM |
| **23709C>T** | T716I | S | HET | HOM |
| **24506T>G** | S982A | S | HOM | HOM |
| **24914G>C** | D1118H | S | HOM | HOM |
| **28111A>G** | Y73C | ORF8 | HOM | HOM |
| **28883G>C** | G204R | N | HOM | HOM |
| **28977C>T** | S235F | N | HOM | HOM |
| **29002C>T** | G243G | N | HOM | HOM |
| **29870C>A** | downstream | N, ORF10 | HET | HET |

Nsp: non-structural proein; ORF: open reading frame; S: Spike protein; N: Nucleocapsid gene; HOM: homogenous; HET: heterogenous

Homogenous: all the viral isolates in the sample have the same mutation.

Heterogenous: specific mutation is not present in all the viral isolates in the sample.

performing hand sanitisation.

The neonate was negative for both SARS-CoV-2 genome and anti-SARS-CoV-2 IgG/IgM antibodies on the day of birth and at 48-hour follow-up. The virus was not detected in any of the products of conception. Amniotic fluid was not collected due to the rupture of the foetal membrane. At 48 hours post-birth, the mother was vitally stable with no active complaint with an oxygen saturation of 97% on room air. The chest X-ray of the mother showed no significant lesions, but a patchy shadow was seen in the right lung field. On day 7 postpartum, both Open Reading Frame 1ab or ORF1ab and Nucleocapsid or N genes of SARS-CoV-2 were detected in both neonate (CT=17) and mother (CT=34) nasopharyngeal swab samples showing high viral load in the neonate's sample. The baby was seronegative for both IgM and IgG, and was completely stable.

The viral RNA, isolated from the subject and her neonate on day 7 follow-up specimens, was subjected to deep genome sequencing. Through the alignment with the SARS-CoV-2 reference genome (NC_045512), 26 mutational sites were detected in the mother and the newborn's samples (Table 3).

The viral genomes of the study clustered close to the clades of SARS-CoV-2 genomes from England, as demonstrated by the phylogenetic relationship between the sequenced genomes and SARS-CoV-2 whole-genome sequences from 18 countries (Figure 2). The phylogeny confirmed the strain of the virus as the Alpha variant as deduced from the PANGOLIN lineages analysis. The genome sequence was uploaded to the National Centre for Biotechnology Information (NCBI) GenBank under accession number MZ5627071[5].

## Discussion

The current study, to our knowledge, is the first comprehensive research providing substantial evidence on the timing of mother-to-child transmission of SARS-CoV-2 in Pakistani pregnant cohorts through multiple testing at different time points and the analysis of various specimens for viral genome detection.

Irrespective of the vaccination status, the study observed a mild to moderate course of COVID-19 infection among the infected cases. Contrary to what has been reported[16], no maternal mortality or increased need for intensive care unit (ICU) was observed in these pregnant women. This is noteworthy considering that 66.7% of maternal deaths were reported during the 4th wave of the pandemic from Bahawalpur, Pakistan[17]. No increased risk of preterm births and C-sections was observed in subjects compared to the uninfected pregnant women. A study found no maternal complications and/or loss of pregnancy in the early trimester infections[18].

A meta-analysis discovered 28,952 mothers infected with SARS-CoV-2 from 472 studies conducted between December 2019 and August 2021[19]. Only 14 of these neonates had their timing of virus transmission known, with 5 of them confirming early postnatal infections. The study found that severe maternal COVID-19 increased the chances of a positive PCR result in newborns[19]. In contrast, one current subject had a minor illness and did not require ICU care, and no maternal mortality was detected.

The current study reported an early postnatal transmission of the virus from the third wave of the pandemic when the Alpha variant, or the B.1.1.7 lineage, was predominant[20]. The Indexed case had a previous history of PPROM at 32 weeks. This time, the preterm

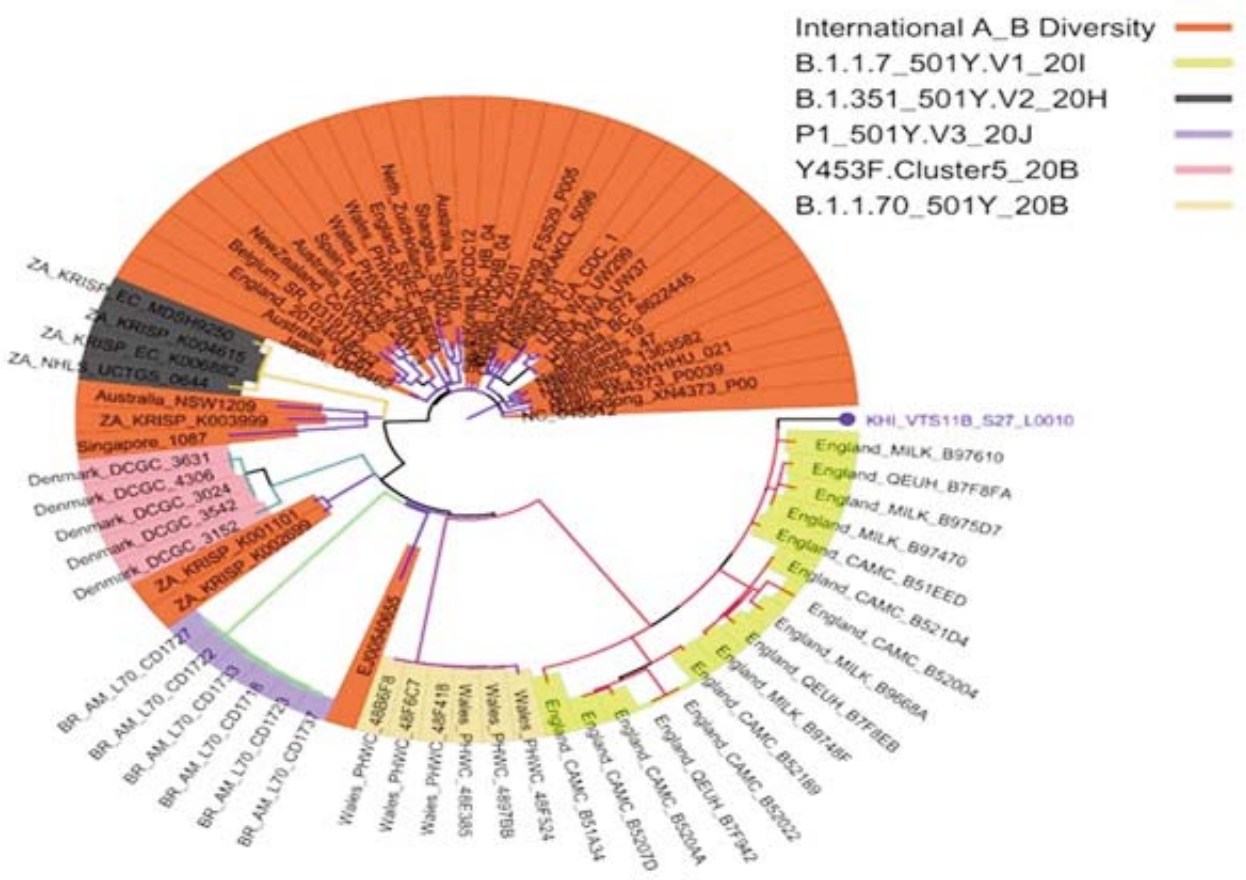


**Figure-2:** Phylogenetic relationship between the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) genome of neonate and SARS-CoV-2 genomes reported from 18 countries.

rupture occurred at 34 weeks of gestation. The preterm birth may not be attributed to COVID-19 infection in this case. The duo was roomed-in together, and the mother was allowed to breastfeed the baby. A multicentre cohort study, assessing the safety of keeping mother-neonate pairs in the same room and breastfeeding, also reported a positive nasopharyngeal swab sample of a baby on day 7 of life[21]. The study indicated that postnatal SARS-CoV-2 transmission from mother to infant was uncommon but a possibility. In the current case, there was a 7-day gap between the commencement of nursing (April 13, 2021) and the baby's positive nasopharyngeal sample (April 20, 2021). This indicated early postnatal transmission of the virus from mother to baby when they were roomed-in[22]. The virus was not transmitted through breastmilk since no viral genome was found in the milk[23].

The current study did not find any evidence of in-utero or intrapartum exposure of neonate to the virus as the baby remained seronegative and also RT-qPCR negative from birth till 48 hours of life. Similarly, placental tissue and sterile specimen i.e., neonatal serum, also showed no viral presence. However, on day 7 of life, the neonate tested positive for the virus with a high cycle threshold value (CT=17). All these findings correlated with the definition of early postnatal transmission of SARS-CoV-2, as defined by the World Health Organisation (WHO) scientific brief[24].

The major strength of the current study was its prospective design and a significant sample size. However, the study had its limitations as well as the sample was raised from within a single hospital that may affect the external validity of the findings. Also, the unusual occurrence of neonatal infection, detected in only 1 of the 11 newborns, may have an impact on the broader implications about vertical transmission. The brief neonatal follow-up period also made it difficult to assess any long-term complications caused by the infection.

## Conclusion

The vertical transmission potential of SARS-CoV-2 in Pakistani pregnant women was found to be low.

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**Conflict of Interest:** None.

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