American Journal of Medicine and Public Health

Research Article
Published: 21 Jun, 2021

# SARS-CoV-2 Virus in Breast Milk and Vaginal Swabs among Pregnant and Postpartum Women with Covid-19 – A Study from a Tertiary Care Hospital in Chandigarh, India

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## Abstract

**Background:** To test SARS-CoV-2 in vaginal swabs and breast milk & to look for inhibitory effects of breast milk on viral RNA isolation.

**Methods:** We tested breast milk and vaginal swabs for SARS-CoV-2 in seven COVID-19 pregnant and postpartum patients. To evaluate the inhibitory effect of breast milk on RNA isolation, breast milk of healthy mothers was spiked with SARS-CoV-2 of known Ct value in dilutions. Four neonates were also tested for SARS-CoV-2.

**Results:** Of the seven patients, one was antenatal in second trimester, four were early postpartum and two were breast-feeding with a child of 3 months and 2 years of age, respectively. All breast milk and vaginal swab samples tested negative for SARS-CoV-2. The serial dilutions of spiked breast samples from healthy lactating mothers when tested by real time PCR showed the increase in Ct value as compared to standard spiked VTM samples. The four neonates were separated from mothers at birth, given replacement feeds, and tested negative at 48 h and day 5 of life. The two breast-feeding children were positive for COVID-19 and were asymptomatic.

**Conclusion:** We observed negative results for SARS-CoV-2 virus in vaginal swabs, breast milk and neonatal swabs suggesting that these two sources may not contribute to vertical transmission. Due to the inhibitory factors present in breast milk, SARS-CoV-2 might not have been detected by present real time PCR assay if viral copy number is too low. However, more literature is needed to elucidate whether SARS-CoV-2 virus can be transmitted through breast milk and vaginal secretions.

**Keywords: Breast milk; COVID-19; Reverse Transcription Polymerase Chain Reaction (RT-PCR); SARS-CoV-2 virus; Vaginal swab; Vertical transmission**

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**Received Date**: *19 May 2021*
**Accepted Date:** *16 Jun 2021*
**Published Date:** *21 Jun 2021*

***Citation:***
*Joshi B, Suri V, Bagga R, Singh MP, Suri V, Goyal K, et al. SARS-Cov-2 Virus in Breast Milk and Vaginal Swabs among Pregnant and Postpartum Women with Covid-19 – A Study from a Tertiary Care Hospital in Chandigarh, India. Am J Med Public Health. 2021; 2(2): 1020.*

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## Introduction

The SARS-CoV-2 pandemic has emerged as an unprecedented challenge to the current medical practice. Various management protocols are being evaluated and modified frequently to address key concerns of maternal and neonatal health. The SARS-CoV-2 virus transmission is through respiratory droplets and direct close contact. Till date, there is no conclusive evidence regarding vertical transmission of SARS-CoV-2 virus through vaginal secretions during delivery or breast milk in the postpartum period [1-3]. To enhance the current knowledge on perinatal transmission of SARS-CoV-2 virus, the authors performed real time Reverse Transcription Polymerase Chain

Reaction (RT-PCR) on vaginal swab and breast milk samples of antenatal and postpartum patients. To assess the inhibitory effect of breast milk components on RNA isolation, breast milk from healthy lactating mothers was spiked with the SARS-CoV-2 sample of known Ct value in serial dilutions.

## Methods

This study was carried out in the Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, a tertiary care hospital in Northern India. During the ongoing COVID-19 pandemic, one building of this institute was converted into a dedicated COVID hospital. From May 1st, 2020 to May 31st, 2020, seven COVID-19 positive patients (one antenatal, six postpartum) were admitted to the dedicated COVID building of the institute. We tested breast milk and vaginal swabs for SARS-CoV-2 virus in these patients. Permission for the present study was taken from the ethics committee of the institute.

These seven patients were diagnosed to have SARS-CoV-2 by RT-PCR in nasopharyngeal swab samples which were tested in the Department of Virology. The antenatal patient was diagnosed to be COVID-19 positive during community screening. The four postpartum patients had been delivered in a nearby local hospital at which time their COVID-19 status was not known and were admitted to PGIMER after they were diagnosed to be COVID-19 positive. Two breastfeeding women and their children (age 3 months and 2 years, respectively) were COVID-19 positive. An informed consent was obtained from these patients for participation in the present study.

Detailed epidemiological and clinical characteristics along with neonatal outcome were noted. Samples of breast milk (except in the antenatal patient) and vaginal swabs were collected and tested for SARS-CoV-2 virus using real time PCR kits as per the manufacturer instructions. All neonates were isolated at the local hospital where they had been delivered and were not transferred with the mother to the present hospital. The nasal swab and oropharyngeal swab of the neonates were also tested for SARS-CoV-2.

In order to evaluate the inhibitory effect of breast milk components on RNA isolation, breast milk from three healthy lactating mothers was collected and spiked with the SARS-CoV-2 sample of known Ct value in serial dilutions as shown in Figure. As a control, the same positive sample was spiked in the Viral Transport Medium (VTM) as a reference standard (Hi media, India). RNA was extracted from both the samples using QIAamp Viral RNA Mini Kit (Qiagen, Germany). Isolated RNA was subjected to qRT-PCR kit by LabGun™ COVID-19 assay (LabGenomics, Korea). Student t test was applied for statistical significance using Graphpad prism software version 8.4.2.

## Results

Table 1 describes clinical and epidemiological characteristics of all patients. All seven patients were residing in a containment zone of the city and had a history of contact with COVID-19 patients. One patient was antenatal in her early second trimester and four were in the postpartum period. There were two breast feeding mothers who were admitted along with their child. The age of the patients ranged from 19 to 36 years and none of them had any coexisting morbidities like diabetes, hypertension or heart disease. Five patients were asymptomatic and two had mild symptoms for COVID-19 in the form of fever and cough. All seven patients remained stable throughout their hospital stay and were discharged after testing negative. The antenatal patient had a normal viable fetus of 12 weeks gestation on ultrasound of the four postpartum patients, three had undergone emergency caesarean due to obstetrical indications (patients number 2 to 4) and one delivered vaginally (patient number 5). All four newborns had a good apgar score at birth and were separated at birth from mother. The neonates were cared for by a healthy family member in isolated rooms. They tested negative at 48 h and day 5 of life and discharged in a healthy condition on day 6 of life (Figure 1).

Lab parameters showed moderate to severe anemia in three (Patient number 2 to 4), elevated C-reactive protein in two (Patient

**Table 1:** Epidemiological & clinical characteristics of COVID positive patients.

| Patient no. | Age (years) & parity | Date of admission & Clinical stage of COVID-19 | Obstetric profile at admission | Lab parameters | Epidemiological history |
|---|---|---|---|---|---|
| 1 | 22<br>Gravida 2, para 1 | 5/5/20<br>Mild | Pregnant, 13 weeks period of gestation | Hb=11.8g/dl, TLC=6000/mm$^3$,Plt=1.4lac/µL<br>LFTS= Normal, RFT=Normal<br>D dimer=262ng/ml ,Vit D3=8.95ng/ml ↓<br>CRP=5.9mg/L, TSH=0.4Iu/ml | Husband & son positive for Covid 19 |
| 2 | 24<br>Para 1 | 8/5/20<br>Asymptomatic | Day 1 Post emergency caesarean | Hb = 7.8 g/dl,TLC=9400/mm$^3$,<br>Plt 1.3 lac/µL<br>LFTS/RFT/ Serum electrolytes= normal<br>D dimer-1110ng/ml ,Fibrinogen=8.5gm/L<br>CRP=226mg/L↑, Vit D3=6ng/ml↓<br>Pro BNP=305pg/ml | Residing in containment zone |
| 3 | 19<br>Para 1 | 2/5/20<br>Asymptomatic | Day 2 Post emergency caesarean | Hb=8.4g/dl, Microcytic, TLC=12000Plat=97,000 lac/µL<br>LFT/RFT/HbA1c/ electrolytes=normal<br>Vit D3=3.0ng/ml↓ , D dimers=3.14µg/ml↑ | Residing in containment zone |
| 4 | 29<br>Para 2 | 25/5/20 Asymptomatic | Day 2 Post emergency caesarean | Hb=5.5g/dL,TLC=6400/mm$^3$,Plt=2.3lac/ µL,PTI=93%,LFT,RFT=normal, CRP=44.61 mg/L,Fibrinogen=6.3gm/L<br>DDimers=818ng/ml | Residing in containment zone |
| 5 | 26<br>Para 1 | 8/ 5/20<br>Mild | Day 2 Post vaginal delivery | Hb=11.8g/dL,TLC=7900/ mm$^3$<br>Plt=1.69lac /µL,LFTS=normal<br>Vit D3=10.5ng/ml, D dimer=750ng/ml, | Residing in containment zone |
| 6 | 36<br>Para 1 | 10/5/20 Asymptomatic | Breastfeeding 3 months old child | Hb= 10.5g/ dl,TLC=6000/ mm$^3$,<br>Plt=1.8lac/ µL, LFTs,RFT=normal<br>D dimer=246ng/ml | Husband & Brother in law positive for Covid 19 |
| 7 | 32<br>Para 2 | 10/5/20 Asymptomatic | Breastfeeding 2 years old child | Hb=11.5g/ dL,TLC=4300/ mm$^3$<br>Platelets=2.5lacs,d dimer=360ng/ml,<br>LFTs, RFT=normal<br>Vit D3=6ng/ml | Husband & Sister in law positive |

TLC: Total Leukocyte Count; Plt: Platelet Count; LFTs: Liver Function Tests; RFT: Renal Function Tests

**Table 2:** COVID-19 testing results of breast milk, vaginal swabs & neonate/child.

| Patients Number | Date of diagnosis of COVID-19 | Date of breast milk & vaginal swab sampling | Interval between diagnosis of COVID &sample of breast milk & vaginal swab | SARS-CoV-2 in breast milk | SARS-Cov2 in vaginal swab | COVID status of neonate/child and age |
|---|---|---|---|---|---|---|
| 1 | 5/5/20 | 9/5/20* (vaginal swab only) | 4 days | ------- | Negative | ----- |
| 2 | 8/5/20 | 9/5/20 | 1 day | Negative | Negative | Negative at 48 hours &day 5 of life |
| 3 | 2/5/20 | 9/5/20 | 7 days | Negative | Negative | Negative - at 48 hours & day 5 of life |
| 4 | 25/5/20 | 26/2/20** | 1 day | Negative | ----- | Negative - at 48 hours & day 5 of life |
| 5 | 8/ 5/20 | 9/5/20 | 1 day | Negative | Negative | Negative - at 48 hours & day 5 of life |
| 6 | 10/5/20 | 12/5/20 | 2 days | Negative | Negative | Infant positive 3 months old |
| 7 | 10/5/20 | 12/5/20 | 2 days | Negative | Negative | Child positive 2 years old |

*Pregnant patient
**Did not consent for vaginal swab

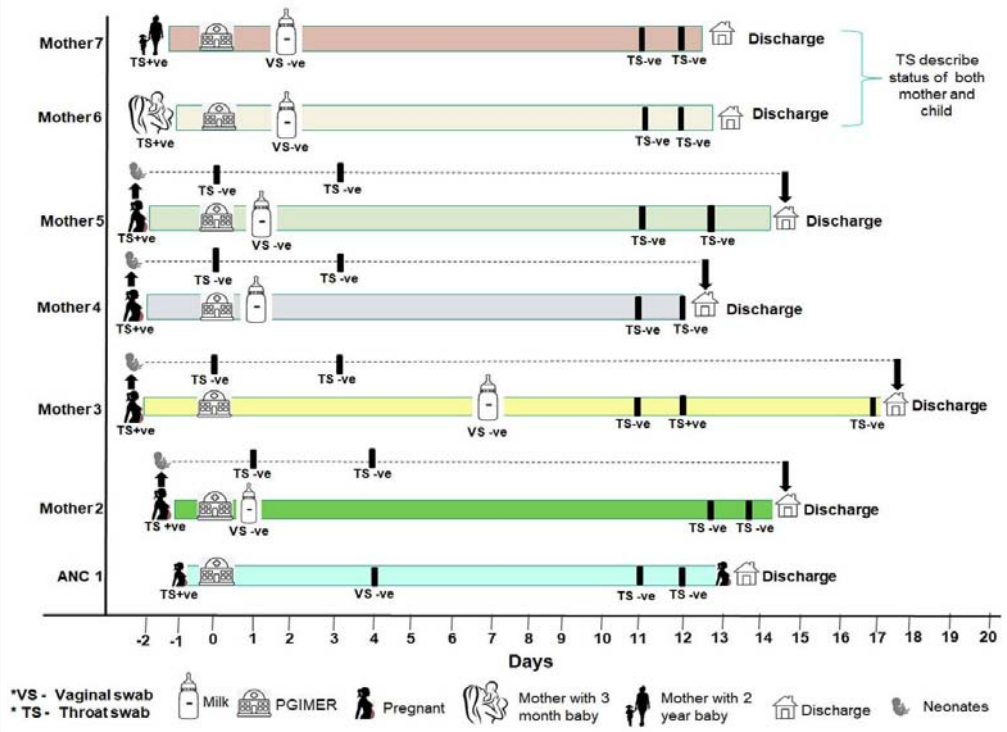


**Figure 1:** Timeline events of various mothers & neonates showing breast milk & vaginal swab testing.

number 2 and 4) and decreased level of vitamin D3 in five patients (numbers 1,2,3,5,7). One patient was severely anemic (number 4) and was given two units of blood transfusion. The cause of anemia was not related to COVID-19 disease and was pre-existing. Peripheral smear showed a dimorphic picture suggesting that it was due to nutritional deficiency. Figure depicts COVID-19 test results in breast milk, vaginal swabs and throat and nasal swabs of neonates/child. The presence of SARS-CoV-2 virus was tested in breast milk of six (patient numbers 2 to 7) and vaginal swabs of six (patient numbers 1-3 and 5-7). The time interval between diagnosis of COVID-19 and sampling varied from one to seven days. None of the breast milk or vaginal swab samples tested positive for the presence of SARS-CoV-2 virus. There was no evidence of COVID-19 infection in the swabs of the four neonates. The two children (age 3 months and 2 years, respectively) tested positive and were admitted to the hospital along with their mothers.

Figure 2A, 2B shows the effect of breast milk on detection of SARS-CoV-2. The serial dilutions of spiked breast samples obtained from 3 healthy lactating mothers when tested by real time PCR showed the increase in Ct value as compared to standard spiked VTM samples. Thus, it is likely that there may be inhibitory factors present in breast milk which may result in an increase in Ct value for the detection of SARS-CoV-2.

## Discussion

Infection with other Corona viruses like MERS-CoV and SARS-CoV during pregnancy was associated with life threatening critical illness in the mother and increased risk of abortion, fetal growth restriction and still birth [4,5]. The current pandemic due to SARS-CoV-2 virus has limited literature regarding COVID-19 infection during pregnancy. Available data suggests that most of the cases are asymptomatic or have mild symptoms only and there is no evident adverse outcome in the form of increased maternal and fetal morbidity and mortality [2,6,7]. This observation is in concurrence

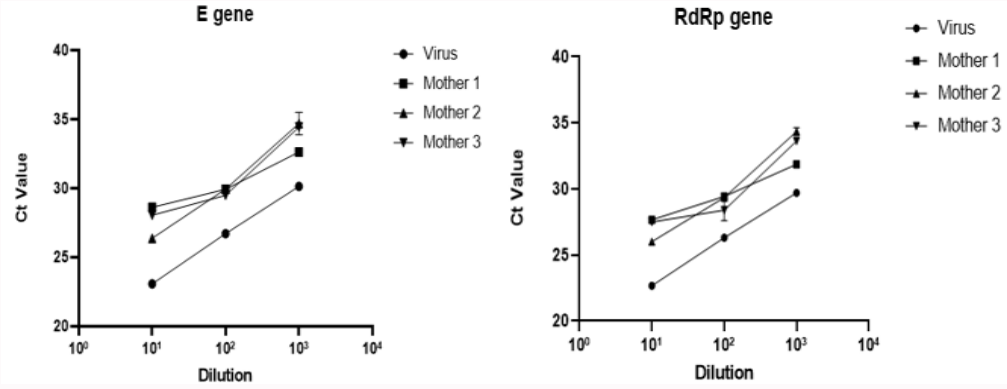


**Figure 2A & 2B:** Cycle threshold (Ct) values among spiked breast milk as compared to control values in spiked Viral Transport Medium (VTM).

with the present study where all seven patients with COVID-19 infection were asymptomatic or had mild disease and remained stable throughout their hospital stay. An important concern about COVID-19 infection in pregnancy is the risk of SARS-CoV-2 virus transmission from the mother to the fetus during pregnancy, labor and delivery or in the postpartum period. There is a fear that the fetus may get infected due to contact with vaginal secretions during its passage through the birth canal, hence many obstetricians chose to deliver these women by a caesarean section without a clear medical indication [1,2,14]. However, studies reported till dates have not been able to demonstrate the presence of SARS-CoV-2 virus in vaginal secretions. Therefore, there is an ongoing debate regarding the need to perform caesarean section to reduce vertical transmission and the safety of vaginal delivery in women with COVID-19 infection [2,3,6,8]. Another issue has been raised regarding the safety of breast feeding in these women. Breast milk is a rich source of nutrients and has immunological benefits for the neonate. There is no clear evidence regarding transmission of SARS-CoV-2 virus through breast milk of mothers with COVID-19 disease. In most of the pregnant women testing positive for SARS-CoV-2 virus, vaginal secretions and breast milk specimens have tested negative [2,3,6,9,10]. Breast milk samples of ten mothers tested by Liu et al were found negative for this virus [11]. An early study tested breast milk of three postnatal mothers and throat swabs of eight newborns; no viral RNA was detected in any milk or throat sample [12]. Till date, there are only three cases reported with breast milk positive for SARS-CoV-2 virus [13-15]. Rüdiger et al. (Ulm University, Germany) examined milk from two breast feeding mothers infected with SARS-CoV-2 and detected viral RNA in breast milk of one mother for four consecutive days [13]. She was breast feeding her neonate with all necessary precautions but the neonate also tested positive for the virus [13]. However, whether the newborn was infected by breastfeeding or by any other mode of transmission was unclear [13]. To assess the safety of breast feeding and vaginal birth, a cohort of thirteen SARS-CoV-2 infected pregnant women (five in the first trimester, three in the second trimester and five in the third trimester) was studied by Wu et al. from Wuhan, China [14]. Of the five women in the third trimester, one delivered vaginally and four by caesarean section. Breast milk samples of three women were tested for SARS-CoV-2 virus, one on day one postpartum, one on day 6 and one on day 27. Breast milk sample collected on day one postpartum was positive for SARS-CoV-2 virus and became negative when re-tested on day 3 postpartum. The breast milk samples of the other two women were negative for SARS-CoV-2 virus. SARS-CoV-2 virus was negative in neonatal throat and anal swabs on day 1 and 3 in all 5 neonates [14]. No viral RNA was detected in vaginal secretions of these thirteen women indicating that vaginal delivery can be a safe option. The third case was one report of probable congenital SARS-CoV-2 infection in a neonate [15]. The mother had active COVID-19 disease and her breast milk, vaginal swab and placental tissue showed the presence of SARS-CoV-2 virus. The neonate was separated from the mother immediately after birth and tested positive at birth, on day 5 and day 7 of life [15]. The congenital infection of SARS-CoV-2 virus in the neonate was suspected on the basis of the fact that the neonate was born by caesarean section prior to membrane rupture, the neonate was separated from the mother since birth and placenta had SARS-CoV-2 virus [15].

This study was undertaken to find out the evidence of vertical transmission 7 to look for factors in breast milk which may hinder detection RNA of SARS-CoV-2 virus. The test results are in accordance with previous studies which showed no evidence of vertical transmission through vaginal secretions and breast milk [2,3,6] & now enough literature has confirm the same [16]. To conclude, the present data may add to the existing literature supporting the absence of vertical transmission via vaginal delivery or breast feeding. The safety of breastfeeding is now established by larger studies, mothers with COVID-19 is given an informed choice about continuing to breast feed or to avoid breastfeeding until testing negative. As this infection lasts two to three weeks only, it may not deprive the neonate of most of the benefits of breast milk. In case they choose not to breast feed during infection, they are advised to express breast milk to sustain lactation, as was done in four patients in the present study.

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