

Investigation of SARS-CoV-2 RNA in Milk Produced by Women with COVID-19 and Follow-Up of Their Infants: A Preliminary Study

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Abstract

Objectives: Studies have shown that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is primarily transmitted from person to person via airborne droplets. It is unclear whether it can be shed into human milk and transmitted to a child via breastfeeding. We investigated the presence of SARS-CoV-2 RNA in human milk samples of 15 mothers with coronavirus disease 19 (COVID-19) and in the throat swab samples of their infants.

Methods: This is a prospective observational study in which breast milk samples were collected from 15 mothers with COVID-19. The presence of SARS-CoV-2 RNA in the whole human milk samples of the patients was investigated using RT-qPCR. All of the infants underwent a clinical follow-up during their 14-day isolation and their throat swab samples were tested for SARS-CoV-2 RNA.

Results: Of 15 mothers with COVID-19, SARS-CoV-2 RNA was detected in milk samples from 4 mothers. The throat swab samples from these mothers' infants were found to be positive for SARS-CoV-2 RNA. Three of the four mothers were breastfeeding. In addition, during the 14-day isolation, all but three of the mothers breastfed their infants. Of the 12 breastfed infants, while the test for SARS-CoV-2 RNA in throat swab samples was negative in six of the infants, the other six infants, who had mild COVID-19 symptoms, tested positive for SARS-CoV-2 RNA. Clinical outcomes of all mothers and infants were uneventful.

Conclusion: To our knowledge, this is the first case series with the largest number of cases with SARS-CoV-2 RNA positivity in human milk samples of mothers with COVID-19. However, we believe that the benefits of breastfeeding may outweigh the risk of SARS-CoV-2 infection in infants.

Keywords: COVID-19; human milk; breastfeeding; infant; SARS-CoV-2; SARS-CoV-2 RNA

What's already known about this topic?

It is unclear whether SARS-CoV-2 can be shed into human milk and transmitted to a child via breastfeeding. To date, there is limited published literature on this issue. In previously reported cases of human milk testing positive for SARS-CoV-2, environmental contamination or retrograde flow from an infected infant could not be ruled out.

What does this article add?

SARS-CoV-2 RNA was detected in human milk samples from 4 of 15 mothers with COVID-19. To the best of our knowledge, this is the first case series with the largest number of cases with SARS-CoV-2 RNA positivity in human milk samples of mothers with COVID-19. These findings suggest that SARS-CoV-2 can be shed into human milk. In addition, since one of the four mothers with a milk sample positive for SARS-CoV-2 RNA was not breastfeeding, the risk of transmission from the infant to the mother's milk was ruled out.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes an extremely infectious disease. The World Health Organization (WHO) has declared the current outbreak to be a global public health threat. Studies have shown that SARS-CoV-2 is primarily transmitted from person to person via airborne droplets.^{1,2,3} However, it can also be transmitted through skin and ocular surface contact as well as via fecal–oral transmission.^{2,3} The gold standard diagnostic method for coronavirus disease 19 (COVID-19) is the detection of SARS-CoV-2 in nasopharyngeal swab samples using real-time reverse transcription-polymerase chain reaction (RT-qPCR).^{1,2,3} The virus can also be detected in the bronchoalveolar lavage fluid, sputum, saliva, feces, and urine.⁴

It is unclear whether SARS-CoV-2 can be shed into human milk and transmitted to a child via breastfeeding. Thus, we investigated whether SARS-CoV-2 RNA was present in milk samples from fifteen mothers with COVID-19 and in their infants' throat swab samples. In addition, mothers and infants were followed up during 14 days of isolation.

METHODS

Study Design

This was a prospective observational study. All lactating women with COVID-19 and their infants who were admitted to Turgut Ozal Medical Center of Inonu University and Malatya Training and Research Hospital in eastern Turkey between 11 March 2020 and 31 January 2021 were included. This study was approved by the Ethical Committee of Inonu University and the health authority of our country (Ministry of Health, Republic of Turkey), and written informed consent was obtained from each mother.

All cases of COVID-19 were diagnosed by RT-qPCR assays of throat swabs, in accordance with updated guidance.^{3,5} Chest computed tomography (CT) scans were performed in patients with appropriate indications. We collected clinical records, breastfeeding history, and laboratory and radiological findings related to SARS-CoV-2 of 15 mothers and their infants during their hospital stay and during the 14-day isolation after discharge.

Human Milk Sample Collection

Human milk collection was performed with a dedicated breast pump in 9 of the mothers and with manual expression in 6 of the mothers. All of the mothers were advised to follow strict hygienic rules according to international recommendations.^{4, 5} During expression, the mothers wore a surgical mask, and they cleaned their hands with water and soap for at least 15-20 seconds prior to expression. Iodine was used to disinfect the breasts of all patients before milk collection. Samples were stored in sterile containers. Because case 1 had nipple retraction and adhesions, milk sample collection from case 1 using a manual pump required immense effort by the nurse. The nurse wore personal protective equipment to help prevent contamination. All milk samples were examined for the presence of SARS-CoV-2 RNA on the day of sample collection; the milk was not frozen before the analysis. Seven mothers (cases 1, 2, 4, 9, 12, 13, and 15) expressed their milk in the hospital ward where they were hospitalized, while the

others expressed it at home. In mothers whose first milk sample had negative PCR results, a second milk sample was taken 24 hours later. Thus, a total of 26 milk samples were obtained from 15 mothers.

Detection of SARS-CoV-2 in Human Milk by RT-qPCR

On the day each sample was collected, the whole human milk samples were examined for the presence of SARS-CoV-2 RNA at the microbiology laboratory of our center by a team of experts. The presence of SARS-CoV-2 RNA in the patients' milk samples was investigated with RT-qPCR using the Bio-Speedy RT-qPCR kit (Bioeksan R&D Technologies Ltd., Turkey), which targets the virus's RNA-dependent RNA polymerase gene. Viral RNA was extracted using the Bio-Speedy vNAT kit (Bioeksan R&D Technologies Ltd., Turkey), and amplification was performed using a Rotor-Gene Q 5plex HRM device (Qiagen, Belgium). Before testing the milk samples, the analytical methods were tested for use in human milk. Positive and negative controls, which were produced by the manufacturer and supplied in the RT-qPCR kit, were used for all samples. Cycle threshold values of all samples are shown in Table 1.

RESULTS

The main clinical features of the patients in the case series and the results of the analysis of the presence of SARS-CoV-2 RNA in milk and throat samples are shown in Table 1. The age range of the mothers and infants was 24–44 years and 1–300 days, respectively. All mothers were symptomatic at the time of the test for SARS-CoV-2 RNA. The mothers provided either 1 and 2 human milk samples, with a total of 26 obtained at varying time points after their positive throat SARS-CoV-2 RNA tests. SARS-CoV-2 RNA was detected in milk samples from four mothers (cases 1, 11, 12, and 13). Three of the four mothers with SARS-CoV-2 RNA detected in their milk samples were breastfeeding, while one of these mothers (case 1) was not able to breastfeed, as the nipples were retracted. The three breastfeeding mothers continued to breastfeed their infant without separating from them during the 14-day isolation. During the 14 days of isolation, as well as during hospitalization, all but three of the mothers (cases 1, 2, and 15) breastfed their infants with appropriate precautions for infection prevention and control (Table 1). In addition, they did not separate from their infants. Case 2 was taking lithium carbonate, quetiapine, and haloperidol decanoate to manage her bipolar disorder and had not been breastfeeding for the week prior to sample collection due to the use of these drugs. If case 15, she was not breastfeeding due to her twin newborns being in the intensive care unit (ICU). She expressed human milk, and provided it to her newborns.

In our study, the infants (infants 3, 5, 6, 8, 9, and 10) of six of the mothers who breastfed their infants during the 14-day isolation did not develop any symptoms related to COVID-19; in addition, their throat swab SARS-CoV-2 RNA tests were also negative (Table 1). Another six infants who were breastfed by their mothers (infants 4, 7, 11, 12, 13, and 14) developed mild COVID-19 symptoms during the 14-day isolation, and SARS-CoV-2 RNA was detected in their throat swab samples. Those infants were declared healthy.

Case 15

This mother was a 31-year-old woman at 33 weeks of gestation. She had a history of gestational diabetes and had hypothyroidism for 2 years. She complained of myalgia and

cough. Her vital signs were stable, with oxygen saturation of 97% on room air. Laboratory investigations revealed that her C-reactive protein concentrations (5.36 mg/dL, normal range 0–0.35 mg/dL) and D-dimer level (4.24 mg/L, normal range 0–0.55 mg/L) were elevated. Other laboratory findings were normal. Her throat swab sample was positive for SARS-CoV-2 RNA. Chest CT showed minimal ground-glass opacities in the bilateral upper lobe (Figure 1). Ultrasound of bilateral lower extremity was normal. She was hospitalized. On the fourth day of hospitalization, she gave birth to twin newborns via a cesarean section. They were taken to the ICU due to respiratory distress and moaning. The twins required supplementary oxygen, but were not intubated. SARS-CoV-2 RNA was detected in the throat swabs of the twin newborns immediately after delivery. The mother and her newborns were declared healthy and discharged on the tenth and twentieth day of hospitalization, respectively. Both the mother and her twin newborns were found to be negative for SARS-CoV-2 RNA at two consecutive throat swabs before being discharged.

DISCUSSION

In this prospective study, we showed that human milk samples from 4 out of 15 mothers with COVID-19 were positive for SARS-CoV-2 RNA. To the best of our knowledge, this is the first case series with the largest number of cases with SARS-CoV-2 RNA positivity in human milk samples of mothers with COVID-19. Of the four cases in whom SARS-CoV-2 RNA was detected in their milk samples, three of the mothers (cases 11, 12, and 13) were breastfeeding their infants, while one mother (case 1) was not able to breastfeed, as the nipples were retracted. To the best of our knowledge, case 1 is the first case in which SARS-CoV-2 RNA was detected in human milk of a mother who was not breastfeeding her infant. In previously reported cases and in our other three cases with milk samples positive for SARS-CoV-2 RNA, environmental contamination or retrograde flow from an infected infant could not be ruled out.⁶⁻¹⁰

Thus far, the number of cases in which human milk was found to be positive for SARS-CoV-2 RNA is limited in the literature. There are very few case series. Of 42 case reports on milk samples from women infected with SARS-CoV-2, viral RNA was detected in 11 samples from 5 women.⁶⁻¹² One of these cases was reported by Groß et al. in *The Lancet*.⁶ In this case, the SARS-CoV-2 RT-qPCR test result was positive for the newborn. Since the mother was breastfeeding her newborn, the virus could have been transmitted to the breast through the secretions of the newborn. In another case, reported by Wu et al.,⁷ an RT-qPCR test for SARS-CoV-2 was not performed for the newborn.⁷ In our case series, the RT-qPCR test was positive for the milk samples from 4 cases and negative in the other 11 cases.

In a study by Liu et al., 10 milk samples were obtained from mothers after their first lactation. These samples were tested for the presence of SARS-CoV-2 RNA using the RT-qPCR test, revealing negative results.⁸ In another study that enrolled 18 women who had confirmed SARS-CoV-2 infection, 1 of the 64 total milk samples obtained from these women had detectable SARS-CoV-2 RNA.¹⁰ The breastfed infants were not tested. In our study, 4 of a total of 26 human milk samples obtained from 15 mothers with COVID-19 had detectable SARS-CoV-2 RNA. SARS-CoV-2 RNA was detected in the throat swabs of all infants whose mothers had SARS-CoV-2 RNA in their human milk samples (infants 1, 11, 12, and 13). All but one of these infants (infant 1) had been breastfed by their mothers. Although the existing

data indicate that transmission of SARS-CoV-2 through human milk is possible, detection of viral RNA by RT-qPCR in body secretions such as human milk does not demonstrate infectivity.

In a previous case series, although SARS-CoV-2 RNA was detected in one human milk sample from a mother with COVID-19, the viral culture of that milk sample was negative.¹⁰ In our study, although SARS-CoV-2 RNA was detected in the throat swabs of some of the breastfed infants whose mothers' milk samples were positive for SARS-CoV-2 RNA (cases 11, 12, and 13), these findings do not show that the presence of SARS-CoV-2 RNA indicates viability of the virus and that human milk may be a source of infection for the infants. We believe that the possibility of transmission of the virus from the mother to the infant through human milk is very low.

Intrauterine or intrapartum transmission of the virus is another concern. Many types of virus, such as herpes simplex virus and HIV, can be transmitted to newborns by intrapartum transmission.^{13,14} Recently, it was reported that the high levels of IgM and IgG antibody in three neonates born to mothers with COVID-19 suggests that the newborns were infected in the uterus.^{15,16} In a group of 31 cases, SARS-CoV-2 RNA was detected in 2 placentas and umbilical cord blood samples and in 1 vaginal swab in women with COVID-19.¹⁷ Conversely, in another case series, the vaginal swabs of 13 pregnant women with COVID-19 were negative for SARS-CoV-2-RNA. In case 15 from our study, the detection of SARS-CoV-2 RNA in the throat swabs of both the twin newborns born from a mother with COVID-19 immediately after delivery via caesarean suggests the possibility of vertical (intrauterine or intrapartum) transmission. However, cord blood and amniotic fluid samples for SARS-CoV-2 RNA were not taken, and intrauterine transmission for this case can only be speculated.

A major mechanism underlying the introduction of SARS-CoV-2 into cells was revealed by the interaction between the viral S protein and angiotensin converting enzyme 2 (ACE2) in the host.^{1, 18} ACE2 receptors are expressed in several organs, including myoepithelial cells of breast tissue and female reproductive organs.^{19,20} This may explain why SARS-CoV-2 may be present in human milk and vaginal swabs.

One of the most important concerns about breastfeeding during this pandemic is whether mothers with COVID-19 can breastfeed their infants. Human milk has numerous advantages for newborns, including passive transmission of antibodies against several microbial diseases.²¹ Recently, two studies reported the presence of IgM and IgG antibodies for SARS-CoV-2 in the milk of mothers with COVID-19, suggesting the possible protective effect of breastfeeding for infants by providing immune protection.^{17,20} While many researchers^{22,23} do not recommend breastfeeding if there is a risk or confirmation of the disease, the Centers for Disease Control and Prevention (CDC) and the WHO recommend that mothers with suspected or confirmed COVID-19 breastfeed, taking appropriate precautions for infection prevention and control.^{3,24}

In our study on breastfeeding, all but three of the mothers (cases 1, 2, and 15) breastfed their infants during their hospitalization and 14 days of isolation, and all of the mothers took appropriate precautions. Six of the breastfed infants (infants 3, 5, 6, 8, 9, and 10) did not

develop any symptoms related to COVID-19 during the 14-day isolation, and their throat swabs for SARS-CoV-2-RNA were also negative. The other six breastfed infants (infants 4, 7, 11, 12, 13, and 14) had mild COVID-19 symptoms, and SARS-CoV-2 RNA was detected in their throat swab samples (Table 1). These infants were declared healthy. Similar to our study, in a recent study evaluating data from mothers with COVID-19, no infants suffering from COVID-19 during follow-up, even though the majority were breastfed.²⁵ Consequently, our findings support WHO and CDC recommendations that mothers with COVID-19 breastfeed their infants.

Limitations of our study include the small sample size, that viral culture was not performed for human milk samples in which SARS-CoV-2 RNA was detected, and that additional examination of maternal and infant samples was not conducted.

Conclusion

To our knowledge, this case series reports the largest cases with SARS-CoV-2 RNA positivity in human milk samples of mothers with COVID-19 thus far and suggests that SARS-CoV-2 can be excreted into human milk. However, these findings do not show that the presence of SARS-CoV-2 RNA indicates viability of the virus and that human milk may be a source of infection for infants. Additionally, based in part on the results of the present case series, we believe that the benefits of breastfeeding may outweigh the risk of SARS-CoV-2 infection in infants. Therefore, in line with the guidelines of the WHO and the CDC, we recommend that mothers with SARS-CoV-2 breastfeed their infants, provided that they wear a surgical mask. Further studies are needed to determine whether SARS-CoV-2 is shed into human milk and whether it can be transmitted to infants through the milk.

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Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Table 1. Clinical Characteristics of the Mothers with SARS-CoV-2 Confirmed by RT-qPCR and Their Infants.

Figure 1. Chest computed tomography (CT) scan of case 15.

It showed minimal ground-glass opacities in the bilateral upper lobe.