

DETECTABLE SARS-CoV-2 IN HUMAN BREAST MILK: A TURKEY EXPERIENCE

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ABSTRACT

Purpose: Breastfeeding is considered the strongest postnatal bond between mother and infant and is the best source of nutrition that affects infant health and development. However, the global COVID-19 epidemic and the lack of understanding of the vertical transmission of SARS-CoV-2 have significantly limited breastfeeding practices. The aim of this study is to investigate the presence of SARS-CoV-2 virus in the milk of breastfeeding mothers with COVID-19 disease and to provide scientific evidence on whether breastfeeding will be safe.

Material and Methods: For this purpose, breast milk samples were collected from 60 women with COVID-19 in the Turkish city of, and analyzed for the presence of SARS-CoV-2 RNA by RT-PCRs.

Results: The result of the analysis, SARS-CoV-2 RNA was not detected in any breast milk. According to the results obtained in this study; that there is no evidence of mother-to-child transmission of SARS-CoV-2 through breast milk and that mothers can safely breastfeed their infants.

Conclusion: We believe that with deeper analysis and comprehensive studies, it will be possible to fully understand whether the SARS-CoV-2 virus is transmitted to infants through breast milk.

Keywords: breast milk, SARS-CoV-2, COVID-19

INTRODUCTION

The Coronavirus-2019 outbreak (COVID-19) is caused by infection with a new strain of coronavirus, and the severe acute respiratory syndrome has been identified as coronavirus -2 (SARS-CoV-2). On March 11, 2020, the World Health Organization (WHO) declared coronavirus disease and today (COVID-19) more than 110 million cases have been reported worldwide (1). The clinical signs of SARS-CoV-2 infection vary from patient to patient. Most infections are asymptomatic, but severe cases such as viral pneumonia may require oxygen therapy, intensive care, and even mechanical ventilation (2). It is not yet

fully understood whether pregnant women, women who have recently given birth or breastfeeding mothers are at higher risk (3). On May 27, 2020, WHO reported a Guidance on Clinical Management of COVID-19, recommending exclusive breastfeeding for at least the first six months, meanwhile taking mandatory precautions to prevent and control infection in newborns of mothers with suspected or confirmed COVID-19 (4). Indeed, families and health professionals are concerned because of the food safety risks associated with feeding infants, especially during such epidemics. In this pandemic, evidence of transmission of SARS-CoV-2 from

Table 1. Information of mothers

	Mean±Std Deviation
Age of mothers (Years)	27.78±5.30
Age of infants (Days)	219.88±231.87
Number of pregnancies for mothers	3.22±2.04
Birth weight of infants (gram)	2899.00±549.44

Table 2. Studies detected SARS-CoV-2 RNA in breast milk

Country	Sample no	Number and percentage of positive samples (%)	Reference
Turkey	1	1 (100)	(8)
Italy	14	1 (7)	(9)
Italy	2	1 (50)	(10)
USA	18	1 (6)	(11)
Italy	2	1 (50)	(12)
Italy	11	1 (9)	(13)
Germany	2	1 (50)	(7)
Mexico	1	1 (100)	(14)
Canada	1	1 (100)	(15)
Italy	1	1 (100)	(16)
Australia	1	1 (100)	(17)
China	3	1 (33)	(18)
China	5	1 (20)	(19)
California	65	6(9.2)	(20)

mother to child had to be evaluated, either through skin contact with breast milk or through sweat or inhalation. In the first cases in the literature review RNA was detected in the breast milk sample. In this case, the mother had negative nasopharyngeal and anal test results for COVID-19 in her newborn because this newborn was fed breast milk substitute, so it was not possible to identify the risk of infection (5). Because of the risk of non-specific symptoms, investigation of whether the virus is passed to the newborn through milk deserves special attention (6). In May 2021, SARS-CoV-2 RNA tested positive in an infected newborn of a mother and in breast milk using the RT-PCR test (7). The prevalence of the agent, which has spread almost all over the world, in breast milk has not yet been determined. In addition, the composition of breast milk varies depending on the period of breastfeeding. For this reason, it will be important to determine whether the risk of virus transmission changes with the stage of breastfeeding (4). Breast milk is necessary during the first 24

months of life for growth and survival, and to protect the health of the baby. The aim of this study is to investigate the presence of SARS-CoV-2 virus in the milk of breastfeeding mothers with COVID-19 disease and to provide scientific evidence on whether breastfeeding is safe.

MATERIAL AND METHODS

Collection and Preparation of Breast Milk Samples

This study was conducted at the Faculty of Medicine, Harran University, Sanliurfa, Turkey. Ethics committee approval of the study was obtained from Harran University Clinical Research Ethics Committee (HRU/21.11.09; 07.06.2021). Sixty breast milk samples collected during the COVID-19 pandemic (September-December 2020) were used for the study. Information about the mothers whose milk samples were collected is provided (Table 1). From each mother, 2 ml milk samples were collected manually under sterile conditions and transferred into sterile falcon tubes (SPINWINTM PP-15 ml). Then, the milk samples were brought to the laboratory in the cold chain and stored at -20 0C until analysis.

RNA Extraction and PCR Analysis

The collected 60 breast milk samples were brought to room temperature before the study. For extraction, 100 µl of breast milk and 100 µl of Viral Nucleic Acid Buffer (VNAT) (Bio-Speedy Bioeksen R&D Tekn. Ltd. Şti) were collected and vortexed for 15 seconds. The vortexed samples were centrifuged (NF 048, Nüve, Turkey) at 13000 rpm for 2 minutes. From each centrifuged mixture, 5 µl was taken from the liquid part of the bottom and 15 µl of the mix (Bio-Speedy SARS CoV-2 Double Gene RT-qPCR Kit; BS-SY-WCOR-307-100, Bioeksen, Turkey) was added to the dispersed plate. The Bio-Speedy SARS CoV-2 Double Gene RT-qPCR Kit uses SARS-CoV 2 (N) SARS-CoV 2(ORF1ab gene) (Cy5), Internal Control (IC) (Rnase P) (FAM) Version 4 FDA approved kit. The PCR protocol was performed at 52°C for 5 minutes, at 95°C for 10 seconds, 40 cycles at 95°C for 1 second, at 55°C for 1 second. 100 µl of sample from the same samples and 35 more wells of 50 µl of VNAT were mixed with samples in a cabinet with biosafety level 2A. The plate using two negative and two positive controls was loaded into the BIORAD CFX-96 Real-time PCR device (ABD).

The positive material for which the standards for quantification are established was randomly selected

Table 3. Studies in which SARS-CoV-2 RNA was not detected in breast milk

Country	Sample no	Number and percentage of positive samples (%)	Reference
Jordan	1	0 (0)	21
China	6	0 (0)	22
Italy	1	0 (0)	23
China	1	0 (0)	24
Italy	1	0 (0)	25
China	6	0 (0)	26
China	1	0 (0)	27
China	1	0 (0)	28
China	2	0 (0)	29
China	12	0 (0)	30
Sweden	1	0 (0)	31
Korea	1	0 (0)	32
Turkey	1	0 (0)	33
Singapore	1	0 (0)	34
China	1	0 (0)	35
China	4	0 (0)	36
China	1	0 (0)	37
China	1	0 (0)	38
China	10	0 (0)	39
China	2	0 (0)	40
China	23	0 (0)	41
China	1	0 (0)	42
Spain	7	0 (0)	43
Switzerland	4	0 (0)	44
USA	18	0 (0)	45
China	1	0 (0)	46
China	16	0 (0)	47
Italy	1	0 (0)	48
Belgium	1	0 (0)	49
Turkey	29	0 (0)	50
Italy	2	0 (0)	51
Netherlands	1	0 (0)	52
Netherlands	1	0 (0)	53
Australia	1	0 (0)	54
China	1	0 (0)	55
China	1	0 (0)	56
China	12	0 (0)	57
China	1	0 (0)	58
China	1	0 (0)	59
China	4	0 (0)	60
Hindistan	30	0 (0)	61
Spain	60	0 (0)	62

from a positive patient sample with high ct detected in the routine study. The selected sample was stored at -800C until the working day and was removed and brought to room temperature prior to the study. 500 µl of the positive sample containing VNAT and 100 µl of

breast milk from a covid negative mother were transferred to an eppendorphe. The vortexed mixture was diluted ½ with VNAT and 6 standards were obtained. Prepared eppendorfs were centrifuged at 13000 rpm for 2 minutes. Each eppendorf was added to the same PCR plate as 15 µl of mixed + 5 µl of sample 3 times, for a total of 18 wells. 6 more wells of 15 µl mix + 10 µl sample from the same eppendorfs were prepared and loaded into the real-time PCR device which contained a negative and a positive control. The quantitation of the first standard was defined as 106, and the quantitation of the other standards was reduced by ½, respectively (8).

RESULTS

The results of the real-time PCR analysis are shown in Figure 1. In addition, Covid 19 Realtime PCR IC (Internal Control) results are shown in Figure 2 and Covid 19 Realtime PCR PC (Positive Control) results are shown in Figure 3. As a result of the realtime PCR analysis, SARS-CoV-2 RNA was not detected in any breast milk. Only quantitative analysis; revealed that the LOD (Limit of Detection) value was 1000 copies/ml. It is thought that the PCR test results of mothers with a positive viral load below this value can not be detected.

Breast milk is considered the best source of nutrition for most infants. However, since the onset of the global COVID-19 pandemic, confusion has arisen about whether SARS-CoV-2 is transmitted to infants through breast milk and whether infected mothers should be temporarily separated from their infants. For this reason, studies have been conducted on this topic in various countries. In some studies, they reported that they detected the RNA of SARS-CoV-2 in breast milk (Table 2), while in some studies they could not detect the RNA of SARS-CoV-2 (Table 3). Based on the results reported in the limited number of studies published so far (Table 2 and Table 3), we can conclude that our knowledge SARS-CoV-2 virus transmission through breast milk is quite inadequate. Because no clear information was reported in these studies. In studies that reported positive results in breast milk samples (Table 3), the number of samples and the number of positive samples were low, the possibility of SARS-CoV-2 RNA transmission to the milk by contact from the sick mother due to carelessness of hygiene rules while taking the milk sample, the mother's coughing. They reported that they found a positive result due to reasons such as contamination of the nipple as a result of contact and

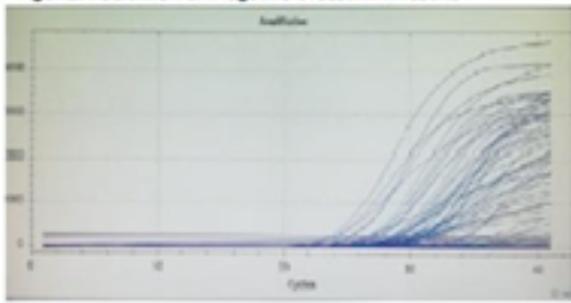


Figure 2. Realtime PCR IC (Internal Control) results

contamination of the nipple and the possibility of transmission of SARS-CoV-2 RNA from the nipple to the milk. In studies where negative results were reported in breast milk samples (Table 3), it was reported that the milk samples were not collected during the viremia period and that the composition of the breast milk might have been affected in inhibiting the virus.

DISCUSSION

In parallel with the results reported in the studies in Table 3, RNA of SARS-CoV-2 was not detected in the breast milk samples we analyzed in this study. According to the results we found, we connect that the SARS-CoV-2 RNA either does not really pass into breast milk or the virus is inhibited from the composition of breast milk. It has been reported in some studies that the composition of milk is effective on viruses. Nolan et al. (2020) reported that antibodies, growth factors and other proteins in the composition of milk have an immunomodulating effect in breast milk (63). Lang et al. (2020) reported that lactoferrin, an iron-binding protein found in breast milk, has an inhibitory effect on SARS virus under cell culture conditions (64). In addition, Bode, reported that proteins such as oligosaccharides (HMOs) in human milk composition have potential antiviral effects (65).

The common result reported in all these studies is that breastfeeding will not be disrupted the health of the baby during this process. However, it is argued that breastfeeding should be continued in a controlled manner. Recommendations for controlled breastfeeding can be listed as follows; If the mother is COVID-19 positive, the mother should wear a mask, practice good hand hygiene and avoid coughing into her chest, and wash her breast with soap and water before breastfeeding. It is said that if breast milk is to be given to the baby by expressing it,

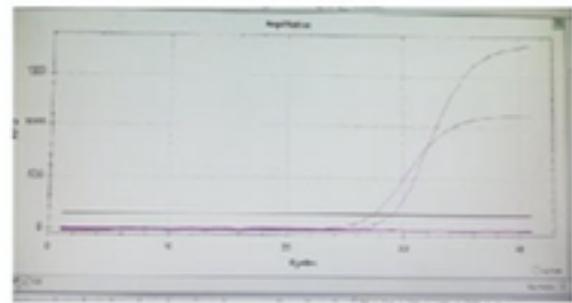


Figure 3. Realtime PCR PC (Positive Control) results

it should follow the same rules and ensure that the milking pump is hygienic.

CONCLUSION

As a result, it is seen that our knowledge about the transmission of SARS-CoV-2 virus through breast milk is quite insufficient. However, according to the results reported in the studies and the results we obtained in our study, it is seen that SARS-CoV-2 RNA may be present at an extremely low rate in human milk. Therefore, we think that it would be a more accurate practice to continue breastfeeding during the Covid 19 disease process, considering the benefits it provides for both the mother and the newborn. We think that care should be taken to prevent the transmission of the SARS-CoV-2 virus from mother to babies by contact during the disease process, and training should be provided by experts to raise awareness of mothers on this issue. We also recommend that infants be given extracted breast milk when direct breastfeeding is not possible due to health concerns. We think that it will be possible with more in-depth analyzes and comprehensive studies to fully understand whether the SARS-CoV-2 virus is transmitted to infants through breast milk.

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