

# SARS-CoV-2 and human milk presence of virus and antibodies in just delivered mothers and possible transmission to the babies: An observational study over a period of 1 year in the state of Mizoram



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## ABSTRACT

**Background:** Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a very virulent and contagious coronavirus and has caused a pandemic of acute respiratory disease, named "Coronavirus disease 2019" (COVID-19) that emerged in the late 2019. In breast milk (BM), presence of SARS-CoV-2 virus and its antibodies have been examined. **Aims and Objectives:** The current observational study was conducted over a period of 1 year in the state of Mizoram to investigate the presence of SARS-CoV-2 virus and antibodies in pregnant mothers and their newborn along with breast milk. **Materials and Methods:** A total of 115 breast milk samples and 70 breast swabs (before or after breast washing) from 115 women who were recently diagnosed with COVID-19 were collected. Samples were analyzed for SARS-CoV-2 RNA using RT-PCR. Breast milk was also analyzed for SARS-CoV2, IgA, and IgG receptor binding domain (RBD), S2 subunit of the spike protein of SARS-CoV-2. **Results:** BM RTPCR was found to be positive 78 and 37 BM samples were found to be negative for RTPCR. Among the infants, 32 were exclusively on breastfeed and 83 were mixed feeding infants. Post-breastfeeding four infants were shown the signs and symptoms of COVID-19. IgA and IgG RBD and S2 subunit of the spike protein of SARS-CoV-2 antibodies were positive in 17, 16, and 12 BM samples which show that maternal antibodies to be developed in BM take time for production and the mothers infected almost 10 days ago, only showed presence of those antibodies. **Conclusion:** Our findings have shown increase admissions of COVID-19 infected antenatal mothers with positive outcome despite the requirement of intensive care. SARS-CoV-2 was detected in BM which was significantly high, but there was no transmission to the babies during the postnatal period.

**Key words:** Breastfeeding; COVID-19; Human milk; Neutralizing antibodies; SARS-CoV-2

## INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a virulent and highly transmissible

beta-coronavirus that emerged in late 2019 and has caused a pandemic of acute respiratory disease, named "coronavirus disease 2019" (COVID-19). In breast milk (BM), the presence of SARS-CoV-2 virus has

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been demonstrated and antibodies against the virus (both IgG and IgA) have also been shown.<sup>1,2</sup> Hence, an observational study over a period of 1 year in the state of Mizoram to know about the prevalence and pathogenic dynamics and transmission to babies for prevention and prophylaxis.

SARS-CoV-2, as virus is found in BM, so it is very necessary by the potential maternal transmission of SARS-CoV-2 by transplacental route, during delivery, and, subsequently, through breastfeeding.<sup>3-5</sup> Some open questions still remain, especially regarding the possibility of finding viable SARS-CoV-2 in BM, although this is not considered a worrying route of transmission.<sup>6</sup> However, in BM, it was pointed out the presence of antibodies against SARS-CoV-2 and other bioactive components that could protect the infant from infection. The aim of study is to investigate the detection of anti-SARS-CoV-2 antibodies in BM of COVID-19 positive mothers and presence of SARS-CoV-2 Virus also in BM.

Evidence is emerging supporting breastfeeding, but there is very less Indian data in this area. This study was done to check the possible transmission of the virus through breastfeeding in the Mizoram, India.

### Aims and objectives

The aim of study is to investigate the detection of anti-SARS-CoV-2 antibodies in breastmilk of COVID-19 positive mothers and presence of SARS-CoV-2 virus also in breastmilk.

## MATERIALS AND METHODS

### Study settings

This study was conducted at tertiary care Medical College in North Eastern India which was functioning as “dedicated COVID-19 hospital” March 2021–2022. This was a prospective observational study to detect presence of SARS-CoV-2 in BM by RT-PCR and clinical outcome of neonates delivered and breast fed by mothers with SARS-CoV-2 infection. Informed consent for participation in the study was taken from expectant mothers, coming for delivery who were positive for the presence of viral RNA in their nasopharyngeal swab (NPS) and BM samples.

### Sample collection for NPS and human breast milk sample

NPS sample was collected from pregnant women admitted for delivery who were tested for SARS-CoV-2 infection at the time of hospital admission and between 48 and 72 h following delivery.

As per protocol of our institute, two samples of NPS of neonates, delivered by the mothers, were investigated for the detection of SARS-CoV-2: one within 24 h of delivery and another after 48 h of delivery.

Breast milk samples collected manually in clean and sterile containers within 3–5 days of delivery were transported in viral transport media to the microbiology laboratory of our institute for the detection of viral RNA through RT-PCR. Samples were directly stored at  $-20^{\circ}\text{C}$ .

The mother-baby dyads were kept together and mothers exclusively breastfed their babies. The use of masks, appropriate hand hygiene, and disinfection of frequently touched surfaces were practiced by the mothers. The healthy mother-baby dyads were discharged after 10 days.

### RNA isolation

RNA was isolated from BM using the Qiagen Viral RNA Mini Kit (Qiagen, #52906) according to the manufacturer’s instructions. The  $200\ \mu\text{L}$  of sample were mixed with  $560\ \mu\text{L}$  lysis buffer (AVL) and incubated for 20 min at room temperature to ensure lysis. Then,  $5.6\ \mu\text{g}$  carrier RNA added to each sample, followed by vortexing and an additional 10 min incubation at room temperature. Ethanol ( $600\ \mu\text{L}$ ) was added to it, vortexed and briefly centrifuged to remove droplets from the lid. The entire volume of lysate was then stepwise loaded onto columns. All subsequent steps were performed as instructed by the manufacturer. Viral RNA was eluted in  $60\ \mu\text{L}$  AVE buffer. The eluted RNA was stored at  $-80^{\circ}\text{C}$ .

### Detection of viral loads using RT-qPCR for SARS-CoV-2 in whole BM

Detection of viral loads using RT-qPCR for SARS-CoV-2 in whole BM was done using WHO recommended primer probe sequence for SARS-CoV-2 *Orf1b* gene followed by confirmation with Imperial Life Science Genes 2 me Viral Detect II RT-PCR for *RdRp* and *E* gene. All the patients’ samples were run along with positive control and a no template control for validation of the run. Real-time RT-PCR was performed in either Biorad CFX96 or ABI Quant studio 5 Dx machine with reaction protocol set as per instruction in the kit insert.

### ELISA method for analysis of antibodies

Human breast milk and IgA and IgG receptor binding domain (RBD), S2 subunit of the spike protein of SARS-CoV-2 by ELISA method as described by Tesini et al.<sup>7</sup> Nunc MaxiSorp 96-well plates (Thermo Fisher, Waltham, MA), were used as per manufacturer’s instructions (pace). Absorbance was read at 405 nm after color development. A weight-based concentration method was used to assign antigen-specific antibody titers in test samples.<sup>8</sup>

## RESULTS

A total of 115 NPS was collected from mothers and their infants along with breast milk from breastfeeding mothers. Their mean age was  $31.2 \pm 5.7$  years. Symptoms such as fever 98, cough 92, and shortness of breath 16 were presented by mothers infected with SARS-CoV-2. Other clinical symptoms were also observed, as given in the Table 1. Among the cases with positives viral RNA detection, one lactating mother was vaccinated with the first dose.

BM sample was tested for SARS-CoV-2 using RTPCR that was detected in 78 samples. Among the infants, 32 were exclusively on breastfeed and 83 were mixed feeding infants. In our finding, a total of four infants presented signs and symptoms of COVID-19 and confirmed by RT PCR during post COVID breastfeeding.

BM samples were analyzed for IgA and IgG RBD, S2 subunit of the spike protein of SARS-CoV-2 antibodies which were positive in 17, 16, and 12, respectively.

## DISCUSSION

In this prospective study, we collected 115 NPS from COVID-19 positive pregnant mothers during the time of admission and later their infants along with human milk also tested them for the presence of SARS-CoV-2. In general, pregnant women more susceptible to infections due to their immunomodulated state and physiological adaptive changes during this period can be lifethreatening.<sup>9,10</sup> Newborn infants are also vulnerable at birth and susceptible to infections also require the best source of nutrition such as human milk. Due to the lack of knowledge of

SARS-CoV-2 and its transmission causes uncertainty, whether breastfeeding should be advised during maternal COVID illness.

We also analyzed the milk for IgA and IgG targeting SARS-CoV-2 and the ability of the samples to neutralize SARS-CoV-2 infectivity. Our study shows the reactivity of the antibodies in BM samples which take time for production during infections. These antibodies developed in BM of infected mothers who might have been infected 10 days prior and only showed presence of those antibodies during investigation.<sup>11</sup>

It was reported that majority of the COVID-19 infected mothers were admitted to the intensive care unit.<sup>12</sup> Clinical characteristics of COVID-19 infected that pregnant mothers were similar to that of COVID-19 other adult infected individual. In the present study, 115 breast milk samples collected from SARS-CoV-2 infected that mother was tested and 78 were positive.

In a study by Perl *et al.*, it was described that secretion SARS-CoV-2 of specific IgA occurs within 2 weeks after vaccination and IgG antibodies occurs after 4 weeks of vaccination.<sup>13</sup> The similar finding was reported by Pace *et al.*, and agrees that specific antibodies present are immunological benefits to the new born infants and is essential to continue breastfeeding during mild to moderate maternal COVID-19 infections.<sup>8</sup>

In some study, breastfeeding was encourage as it provides essential nutrient to the infants while some study lay out appropriate instructions to reduce the risk of infection.<sup>5,14</sup> Some studies proceeded as far as discontinuing breastfeeding and resumed only after the mother was confirmed negative, although those studies did not clarify

**Table 1: Finding of the mothers and the infants in our study**

Maternal Characteristics (n=115)		Infant Characteristics (n=18)	
Age (y)	31.2±5.7	Female	9 (50)
Clinical presentation		Gestational Age (wk)	38.6±1.7
Fever	98 (115)	Birth Weight (g)	3372±560
Cough	92 (115)	Birth Length (cm)	50.3±2.7
Anosmia	34 (115)	Breastfeeding Status	
Dysgeusia	31 (115)	Exclusive	32 (28)
Short of Breath	16 (115)	Mixed Feeding	83 (72)
Body mass index (kg/m <sup>2</sup> )*	28.9±4.8	COVID-19 Diagnostic Test	78
Normal/healthy	101 (115)	Negative Result	37
Overweight	4 (115)		
Obese	10 (115)		
Parity (#)	1.9±1.1		
Cesarean delivery	74 (115)		
Time postpartum (mo)	6.8±7.8		
History of mastitis	1		
Symptomatic at or before enrollment	45		
Developed symptoms after enrollment	70		

that transmission occurs through breastfeeding.<sup>15</sup> In the present study, breastfeeding was ongoing for both positive mothers and infants and was discontinued when either the mother or the infants has to quarantine separately due to either one having critical condition.

A study by Zhu et al., describes the limitation and small sample size of the study but the important factor and matter to be concern was that there is detectable amount of SARS-CoV-2 nucleic acid in human breast milk from lactating mothers.<sup>16,17</sup>

Many studies speculate other path of transmission from COVID-19 infected mothers to infants where amniotic fluid, breast milk, etc., were collected. Despite the efforts, studies have reported that there has not been any strong evidence to suggest development of SARS-CoV-2 infection through vertical transmission.<sup>10</sup> Respiratory droplets still remain as the main route of transmission for SARS-CoV-2.<sup>18</sup>

### Limitations of the study

Due to the small scale of sample and scarcity of data our study was limited requires more investigations.

## CONCLUSION

Our findings have shown increase admissions of COVID-19 infected antenatal mothers with positive outcome despite the requirement of intensive care. SARS-CoV-2 was detected in breast milk which was significantly high, but there was no transmission to the babies during the postnatal period. Although this study was limited and requires more investigation, our findings can provide information for better management of infected pregnant women.

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**FEL**- Concept and design of the study, prepared first draft of manuscript; **ZK**- Interpreted the results; reviewed the literature and manuscript preparation; **LC**- Preparation of manuscript and revision of the manuscript; **SR**- Concept, coordination, statistical analysis and interpretation; **JLR**- Reviewed the literature and manuscript preparation; **FR**- Reviewed the literature and manuscript preparation; **GL**- Reviewed the literature and manuscript preparation; **ZC**- Preparation of manuscript and revision of the manuscript; and **JZ**- Revision of the manuscript.

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