BREASTFEEDING MEDICINE
Volume 00, Number 00, 2024
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DOI: 10.1089/bfm.2023.0323

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Preservation of Anti-SARS-CoV-2 Neutralizing Antibodies in Breast Milk: Impact of Maternal COVID-19 Vaccination and Infection

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Abstract

Objectives: To investigate specific immunoglobulin A (sIgA), specific immunoglobulin G (sIgG), and neutralizing antibodies (NAbs) against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in breast milk and compare immunity in mothers with hybrid immunity (infection and vaccination) versus those solely vaccinated (coronavirus disease [COVID]-naïve).

Methods: A longitudinal study was conducted among lactating mothers who received at least two doses of the coronavirus disease 2019 (COVID-19) vaccine or tested positive for SARS-CoV-2. Details of vaccination and infection were collected through questionnaires and interviews. Fifteen milliliters of breast milk samples, self-collected at 1, 3, and 6 months postvaccination or infection, were sent to analysis for sIgA, sIgG, and NAbs using enzyme-linked immunosorbent assay.

Results: In total, 119 lactating mothers (202 milk samples) were enrolled; 82 participants had hybrid immunity, and 32 were COVID-19-naïve. Two-thirds received a combination of different vaccines and booster shots. Breast milk retained sIgA, sIgG, and NAbs for up to 6 months post-COVID vaccination or infection. At 3 months, mothers with hybrid immunity had significantly higher sIgA and NAbs compared with COVID-naïve mothers (geometric mean [95% confidence interval (CI)] of sIgA 2.72 [1.94–3.8] vs. 1.44 [0.83–2.48]; NAbs 86.83 [84.9–88.8] vs. 81.28 [76.02–86.9]). No differences in sIgA, sIgG, and NAbs were observed between lactating mothers receiving two, three, or more than or equal to three doses, regardless of hybrid immunity or COVID-naïve status.

Conclusion: sIgA, sIgG, and NAbs against SARS-CoV-2 in breast milk sustained for up to 6 months postimmunization and infection. Higher immunity was found in mothers with hybrid immunity. These transferred immunities confirm *in vitro* protection, supporting the safety of breastfeeding during and after COVID-19 vaccination or infection.

Keywords: specific IgA, specific IgG, neutralizing antibodies, SARS-CoV-2, COVID-19, breast milk

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Introduction

Breast milk is widely recognized as the most optimal food for infants. It not only provides essential nutrients for infant growth but also contains a myriad of bioactive components such as hormones, pre/probiotics, and immunoglobulins (Ig) that play a crucial role in promoting infant survival and fostering healthy development.[1,2] Previous research has shown that exclusive breastfeeding for the first 6 months of life and breastfeeding together with complementary feeding afterward are linked to a noteworthy decrease in respiratory and gastrointestinal illnesses during infancy, as well as positive health outcomes later in life.[3–6]

Since the global outbreak of coronavirus disease 2019 (COVID-19), an infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the World Health Organization (WHO) declared a Public Health Emergency of International Concern and global pandemic in early 2020.[7] These had notably impacted peripartum and postpartum clinical procedures, particularly concerning breastfeeding. Research indicates a significant decline in exclusive breastfeeding rates during the lockdown.[8,9] This decrease could be attributable to concerns in potential viral transmission through breastfeeding, safety concerns related to the COVID-19 vaccine, and a lack of support from health care professionals.

Previous studies have identified the presence of antibodies against SARS-CoV-2 in breast milk.[10–14] This discovery suggests a potential transfer of COVID-19 immunity to infants, particularly during the first 6 months of age when vaccination against COVID-19 is not yet available. This protective effect could be attributed to specific immunoglobulin A (sIgA) against SARS-CoV-2, which prevents harmful pathogens from attaching and entering epithelial cells without causing harmful inflammation.[15] Consequently, breastfeeding might offer a natural defense against COVID-19 in early infancy.

However, most previous studies had focused on investigating Ig levels in human milk after individuals received the COVID-19 immunization or experienced an infection. To the best of our knowledge, only a few studies have explored the Ig levels in breast milk from lactating mothers with hybrid immunity (resulting from both vaccination and infection).[16,17] Moreover, there is a limited number of studies that investigate neutralizing antibodies (NAbs), which play a crucial role in the protection conferred by COVID-19 vaccination and/or prior infection. Hence, this study aimed to investigate the levels of sIgA, specific immunoglobulin G (sIgG), and NAbs against SARS-CoV-2 in breast milk from lactating mothers with hybrid immunity. Also, we compared these Ig levels with those of mothers who received only COVID immunization (COVID-naïve) and mothers who had a history of SARS-CoV-2 infection without receiving COVID-19 immunization. Gaining a more comprehensive understanding of how breast milk provides immunity against SARS-CoV-2 could potentially provide reassurance to both mothers and health care providers about the advantages of breastfeeding in these challenging circumstances.

Materials and Methods

Study design and study population

This observational study is a part of the ongoing research project titled "Infant Feeding Survey During the COVID-19 Pandemic" (TCTR20220215012). Data collection was performed from March 2022 to July 2023. Thai lactating mothers were recruited from the postpartum wards of King Chulalongkorn Memorial Hospital in Bangkok, Thailand, as well as various social networks.

To be eligible, participants had to be Thai lactating mothers of healthy, singleton-born infants who had either received at least two doses of the COVID-19 vaccination or had tested positive for SARS-CoV-2 through polymerase chain reaction or antigen test kit within 6 months prior to the collection of breast milk samples. Mothers of preterm infants or infants with congenital diseases were excluded from the study.

This research was granted approval by the Institutional Review Board (IRB) of the Faculty of Medicine, Chulalongkorn University, under IRB No. 956/64.

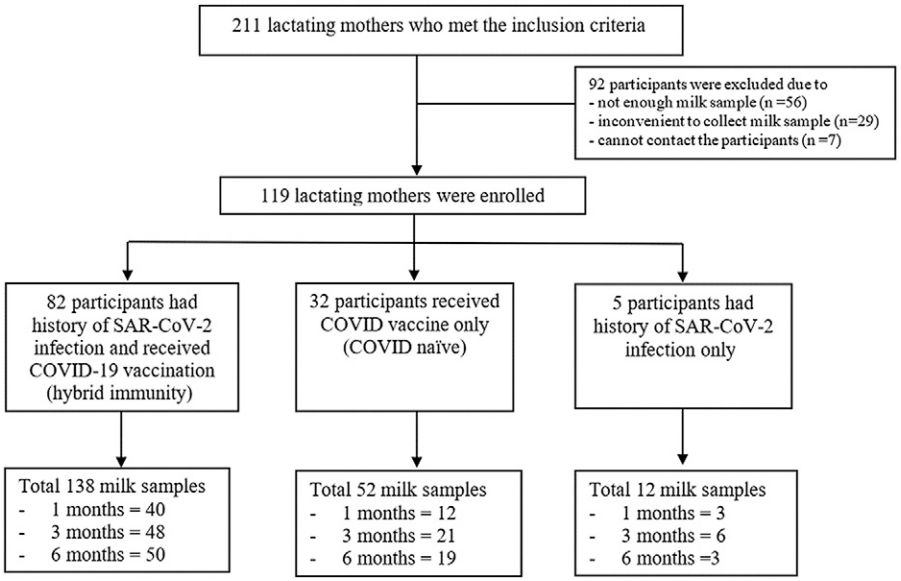

FIG. 1. Details of the lactating mothers and their milk samples.

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Data collection

Demographic data. Demographic information, such as maternal age, pre-pregnancy body mass index (BMI), gestational age, parity, pregnancy complications, family income, and infant details such as gender, birth weight, and delivery method, were obtained by a recruitment nurse. Meanwhile, data on COVID-19 vaccination, including the types, frequency, and timing, or a documented history of SARS-CoV-2 infection were collected through a self-administered questionnaire. To ensure accuracy, all responses were validated through telephone interviews conducted by research assistants.

sIgA, sIgG, and NAbs against SARS-CoV-2 in breast milk. Lactating mothers were instructed to collect 15 mL of breast milk at 1, 3, and 6 months after completing the primary series of COVID-19 vaccination or infection with SARS-CoV-2. If a mother received a booster dose of the COVID-19 vaccine or had the COVID-19 illness after the initial vaccination period, they were asked to provide additional breast milk samples at 1, 3, and 6 months after the most recent event.

The mothers were directed to express their breast milk into a sterile container, which was then stored in their home freezer. The collected specimens were subsequently retrieved by the research team, transported in an insulated box and kept at a temperature of −20°C until they were analyzed for research purposes.

The analysis of sIgA and sIgG antibodies against SARS-CoV-2 in human milk was conducted using the enzyme-linked immunosorbent assay (ELISA) technique with the anti-SARS-CoV-2 ELISA test kit (EUROIMMUN Medizinische Labordiagnostika, Lübeck, Germany). Initially, breast milk samples were subjected to centrifugation at 800 *g* for 10 minutes at 4°C to separate the fat content from the milk. The resulting skimmed breast milk samples were then diluted at ratios of 1:25 for sIgA and 1:10 for sIgG. These diluted samples were pipetted into the reagent wells, together with peroxidase-labeled anti-SARS-CoV-2 sIgA and antibody peroxidase-labeled anti-human sIgG, using an automated ELISA processing system (EUROIMMUN Analyzer I-2P, Lübeck, Germany). Complexes formed between sIgA and sIgG from the milk samples and the respective antibodies during the substrate incubation period. The color developed during this incubation was directly proportional to the concentrations of sIgA and sIgG in the samples. As per the instructions provided by the manufacturer, the semiquantitative test

Table 1. Baseline Characteristics of Lactating Mothers and Their Infants[a,b]

	Total (N = *119*)	*COVID-19 vaccination in conjunction with SARS-CoV-2 infection* (n = *82*)	*COVID-19 vaccination only* (n = *32*)	*SARS-CoV-2 infection only* (n = *5*)	p-*value*
Mothers					
Age (year)	30.9 ± 6.1	30.6 ± 6.1	30.9 ± 5.6	28.4 ± 8.8	0.40
Parity, *n* (%)					0.59
1	52 (43.7)	35 (42.7)	16 (50)	1 (20)	
2	42 (35.3)	31 (37.8)	9 (28.1)	2 (40)	
≥3	25 (21)	16 (19.5)	7 (21.9)	2 (40)	
Pregnancy complications,[c] *n* (%)	53 (44.5)	36 (43.9)	14 (43.8)	3 (60)	0.84
Pre-pregnancy BMI (kg/m^2)	22.5 ± 3.9	22.4 ± 3.9	23.0 ± 4.0	21.3 ± 2.1	0.59
Lactation time (month), median (IQR)	1 (0.5–2)	1 (0.5–32)	1 (1–2)	1.5 (1–2.5)	0.73
Household income (Thai baht), *n* (%)					0.10
<15,000	14 (11.8)	7 (8.5)	6 (18.8)	1 (20)	
15,000–50,000	66 (55.5)	49 (59.8)	13 (40.6)	4 (80)	
>50,000	39 (32.8)	26 (31.7)	13 (40.6)	0 (0)	
Infants					
Male, *n* (%)	55 (46.2)	39 (47.6)	14 (43.8)	2 (40)	0.94
Birth weight (kg)	3.13 ± 0.36	3.13 ± 0.35	3.17 ± 0.41	2.92 ± 0.21	0.36
Mode of delivery, *n* (%)					0.94
Normal vaginal delivery	56 (46.1)	40 (48.8)	13 (40.6)	3 (60)	
Assisted vaginal delivery with forceps or vacuum	3 (2.5)	2 (2.4)	1 (3.1)	0 (0)	
Elective cesarean section	19 (16)	13 (15.9)	6 (18.8)	0 (0)	
Emergency cesarean section	41 (34.4)	27 (32.9)	12 (27.5)	2 (40)	

[a]Values are presented as mean ± standard deviation for continuous variables. Categorical variables are expressed as *n* (%).
[b]Differences in mean and proportion were performed by analysis of variance and chi-square test or Fisher's exact test, respectively.
[c]Pregnancy complications, *n*: anemia, 23; gestational diabetes mellitus, 13; pregnancy-induced hypertension, 9; chorioamnionitis, 3; postpartum hemorrhage, 2; oligohydramnios, 2; hepatitis B infection 2; others (e.g., hyperthyroid, vitamin D deficiency, scoliosis), 8.

BMI, body mass index; COVID-19, coronavirus disease 2019; IQR, interquartile range; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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results were reported as a ratio between the test sample's extinction and the calibrator. The manufacturer's guidelines did not include specific threshold values for breast milk samples.

NAbs against SARS-CoV-2 were determined following the supplier's instructions (EUROIMMUN Medizinische Labordiagnostika). The ELISA technique used microplate strips with wells coated with the recombinant SARS-CoV-2 S1/receptor-binding domain produced via *in vitro* culture of human embryonic kidney 293 cells. Human milk samples were diluted 1:5 in the provided sample buffer containing a biotinylated soluble human angiotensin-converting enzyme 2 (ACE2) receptor, which competes with NAbs in the sample. After a 1-hour incubation, unbound ACE2 molecules were washed away, and a colorimetric reaction was initiated by adding peroxidase enzyme labeled with streptavidin. The color intensity measured at 450 nm inversely correlated with the NAbs concentration in the sample. Results were expressed as the inhibition percentage (%IH) using the formula: %IH = 100% − (Sample Absorbance × 100%/Blank Absorbance).

Statistical analysis

Statistical analyses were conducted using Stata 17.0 (StataCorp., College Station, TX). Before analysis, normality was assessed using histograms and the Kolmogorov test. Categorical data, such as parity, pregnancy complications, family income, mode of delivery, infant gender, details of COVID-19 immunization and infection, were presented as numbers and percentages. Continuous data, including maternal age, gestation, and infant birth weight, were expressed as means with standard deviations. Group differences in means and proportions were evaluated using Analysis of Variance and chi-square tests or Fisher's exact tests, respectively. The geometric mean (GM) and geometric mean ratio (GMRs) with a 95% confidence interval (95% CI) for sIgA, sIgG, and NAbs against SARS-CoV-2 were calculated using two independent sample *t*-tests. All statistical tests were two-sided, and a significance level of $p < 0.05$ was considered statistically significant.

Results

In total, 211 lactating mothers were initially recruited. However, 92 mothers were excluded from the study owing to reasons such as insufficient milk samples (60%), difficulties in collecting milk samples (31%), and loss to follow-up (9%). Consequently, 119 lactating mothers were successfully enrolled, and in total, 202 milk samples were collected. Among these participants, approximately two-thirds had a history of SARS-CoV-2 infection along with COVID-19 immunization, whereas one-fourth were classified as COVID-19-naïve. The details of the participants and milk samples are shown in Figure 1.

Characteristics of lactating mothers

Table 1 presents the baseline characteristics of lactating mothers and their infants. The participants had an average age of 30.9 years. Almost half of them experienced some pregnancy complications, including anemia (43%), gestational diabetes (24%), and pregnancy-induced hypertension (17%). The breast milk collection typically occurred when the infants were around 1 month old. There were no notable differences in maternal age, parity, pre-pregnancy BMI, lactation duration, socioeconomic status, or infant characteristics among the lactating mothers who received hybrid immunity, those who were vaccinated only, and those with a history of only SARS-CoV-2 infection.

Furthermore, the majority of lactating mothers received the initial series of COVID-19 vaccinations. Notably, about two-thirds of them received a combination of different vaccines (mRNA + vector based 47.3%; mRNA + killed virus based 28.4%; vector + killed virus based 6.7%; all types 17.6%), and 63% of them received the booster dose of COVID-19 vaccine. In addition, 73% had prior exposure to COVID-19, but their symptoms were mild. It is noteworthy that most of those who had a history of infection had already been immunized against COVID-19 after the infection (Table 2).

sIgA, sIgG, and NAbs against SARS-CoV-2 in human milk after the vaccination and/or infection

Table 3 and Figure 2 show the comparisons of sIgA, sIgG, and NAbs against SARS-CoV-2 in breast milk following maternal COVID-19 immunization and/or SARS-CoV-2 infection. sIgA, sIgG, and NAbs can be detected in breast

Table 2. Details of COVID-19 Immunization and SARS-CoV-2 Infection

History of COVID-19 vaccination[a]	n *(%)*
Total number of lactating mothers who received complete course of the vaccine	112 (94.1)
Type of the vaccine	
mRNA	20 (17.9)
Vector-based vaccine	14 (12.5)
Killed vaccine	4 (3.6)
Mixed types of the vaccines[b]	74 (66.1)
Total doses of the vaccine	
2 doses	41 (36.6)
3 doses	50 (44.6)
>3 doses	21 (18.8)
History of SARS-CoV-2 infection[c]	
Total number of lactating mothers who had a history of the SARS-CoV-2 infection	87 (73.1)
Having symptoms	74 (85.1)
Vaccination status	
Infection without vaccine	5 (5.7)
Receiving the vaccine before the infection	5 (5.7)
Receiving the vaccine after the infection	77 (88.5)

[a]Brand of COVID-19 vaccine: Pfizer-BioNTech ($n = 15$); Moderna ($n = 5$); AstraZeneca ($n = 13$); CoronaVac ($n = 5$).

[b]Details of mixed types of vaccination: mRNA + vector based ($n = 35$); mRNA + killed virus based ($n = 21$); vector + killed virus based ($n = 5$); all types ($n = 13$).

[c]Method of detecting SARS-CoV-2 infection: antigen test kit ($n = 125$); polymerase chain reaction ($n = 25$).

COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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milk after the mothers had COVID-19 vaccination and/or SARS-CoV-2 infection for up to 6 months. Moreover, lactating mothers with hybrid immunity showed significantly higher sIgA at 3 and 6 months compared with those who received only COVID-19 immunization. In addition, mothers who received both vaccination and infection showed the highest sIgG ratios at 6 months compared with those who had either only vaccination or infection. For NAbs, there were no significant differences among the three groups at 1 month. However, at 3 months, lactating mothers with hybrid immunity had a higher NAbs percentage of inhibition compared with those who received only COVID-19 vaccination. Nevertheless, this difference disappeared at 6 months. Mothers with a history of only SARS-CoV-2 infection exhibited the highest NAbs in breast milk, surpassing the other two groups at both 3 and 6 months.

Subgroup analysis of sIgA, sIgG, and NAbs in breast milk between lactating mothers who received COVID-19 vaccination in conjunction with SARS-CoV-2 infection and those who received COVID-19 vaccination only, according to the number of doses

At 1 month, breast milk sIgA in mothers who received two doses of COVID-19 immunization along with SARS-CoV-2 infection was five times higher than those in COVID-naïve mothers. Similarly, at 6 months, sIgA and sIgG of lactating mothers receiving two doses of COVID-19 vaccination in conjunction with the infection were significantly higher than those of COVID-naïve mothers. However, these differences disappeared when mothers received more than two doses of vaccination. No differences in NAbs were observed at 1, 3, and 6 months between the two groups when classified based on the number of doses (Table 4).

In the within-group comparison, no differences in breast milk sIgA, sIgG, and NAbs were found at 1 and 3 months among lactating mothers who received two, three, or more than three doses, both in mothers with hybrid immunity and COVID-naïve mothers. However, at 6 months, mothers who received three doses of COVID-19 vaccination in conjunction with the infection exhibited slightly lower sIgA and sIgG ratios compared with mothers with hybrid immunity who received two doses of vaccination, whereas their NAbs were slightly higher (GMR [95% CI], sIgA 0.45 [0.21–0.96], sIgG 0.54 [0.3–0.98], NAbs 1.1 [1.01–1.19]). On the contrary, in the COVID-naïve group, mothers who received more than two doses of the vaccine had sIgG ratios at 6 months approximately five times higher than those in the group who received only two doses of COVID-19 immunization (GMR [95% CI], three doses 4.56 [1.86–11.4], more than three doses 5.63 [1.31–23.11]) (Supplementary Tables S1–S3).

Discussion

The COVID-19 pandemic disrupted daily life worldwide, impacting various facets of the global population, including lactating mothers. Prior research indicated a decrease in exclusive breastfeeding rates, potentially attributed to concerns about viral transmission through breast milk. Our recent publication showed that sIgA against SARS-CoV-2

TABLE 3. sIgA, sIgG, AND NAbs AGAINST SARS-COV-2 IN HUMAN MILK AFTER MATERNAL COVID-19 IMMUNIZATION AND/OR COVID-19 ILLNESS[a,b,c]

Period of breast milk collection after the infection/ vaccination	*COVID-19 vaccination in conjunction with SARS-CoV-2 infection*			*COVID-19 vaccination only*			*SARS-CoV-2 infection only*		
	sIgA ratio	*sIgG ratio*	*NAbs (% IH)*	*sIgA ratio*	*sIgG ratio*	*NAbs (% IH)*	*sIgA ratio*	*sIgG ratio*	*NAbs (% IH)*
1 month	2.35 (1.6–3.46)	1.68 (1.3–2.17)	85.35 (83.51–87.24)	1.04 (0.56–1.94)	1.57 (0.83–2.97)	85.3 (83.62–87.02)	1.48 (0.3–7.32)	0.78 (0.02–25.34)	78.79 (53.15–116.8)
3 months	2.72[1] (1.94–3.8)	1.1 (0.86–1.4)	86.83[4] (84.9–88.8)	1.44[1] (0.83–2.48)	1.3 (0.93–1.83)	81.28[4] (76.02–86.9)	2.14 (0.41–11.22)	0.62 (0.07–5.25)	92.1 (89.02–95.28)
6 months	2.06[1] (1.45–2.92)	0.95[2] (0.72–1.26)	85.78 (82.58–89.09)	1.43[1] (0.81–2.53)	0.6[2,3] (0.35–1.03)	86.42[4] (84.99–87.88)	0.54 (0.02–11.8)	0.08[3] (0.01–1.31)	90.63[5] (86.83–94.59)

[a]Values are presented as geometric mean (95% CI).
[b]sIgA and sIgG are expressed as a ratio of the extinction of test sample and calibrator, whereas NAbs were presented as a percentage of inhibition.
[c]The differences of sIgA, sIgG, and NAbs between the groups were performed by independent *t*-tests. Superscripts 1, 2, 3, 4, and 5 indicate specific pairwise differences between the groups.
CI, confidence interval; COVID-19, coronavirus disease 2019; %IH, inhibition percentage; NAbs, neutralizing antibodies; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; sIgA, specific immunoglobulin A; sIgG, specific immunoglobulin G.

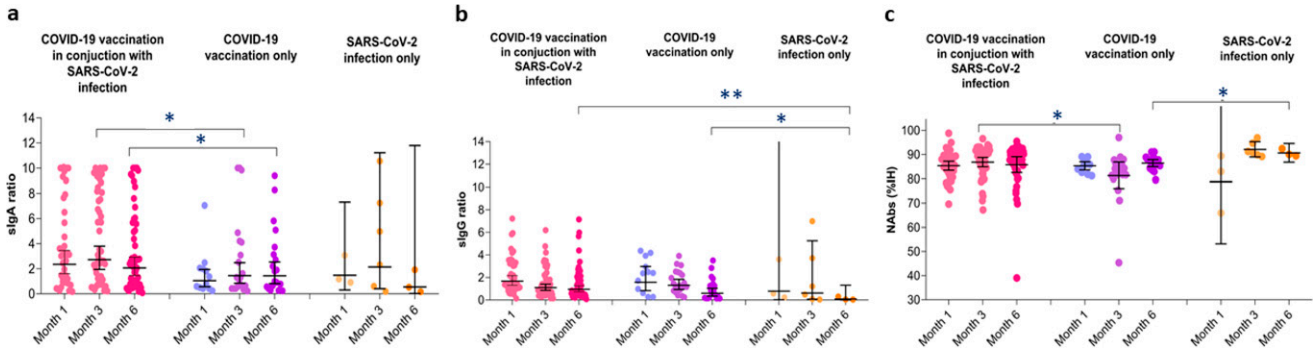

FIG. 2. Comparison of sIgA, sIgG, and NAbs against SARS-CoV-2 in breast milk at 1, 3, and 6 months post-vaccination and/or infection among lactating mothers with hybrid immunity, those receiving vaccination only, and those with SARS-CoV-2 infection only. **(a)**, **(b)**, and **(c)** show the comparison of sIgA, sIgG, and NAbs, respectively. sIgA and sIgG are expressed as a ratio of the extinction of the test sample and calibrator, whereas NAbs are presented as percentage of inhibition (%IH). Error bars show the GM and 95% CI. The asterisks indicate statistical differences by independent *t*-tests (*$p < 0.05$, **$p < 0.001$). CI, confidence interval; GM, geometric mean; NAbs, neutralizing antibodies; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; sIgA, specific immunoglobulin A; sIgG, specific immunoglobulin G.

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can persist in the breast milk for up to 6 months after vaccination.[17] In addition to the sIgA, the finding from this report further revealed a sustained presence of sIgG and NAbs against SARS-CoV-2 in the breast milk of lactating mothers who had a history of SARS-CoV-2 infection and/or had received at least two doses of the COVID-19 vaccination. This strengthens the possibility that these immunities can pass on and offer protection against SARS-CoV-2 infections for their breastfeeding infants.

Our study found the presence of sIgA, sIgG, and NAbs against SARS-CoV-2 for a duration of at least 6 months in breast milk after the mother received the COVID-19 vaccination and/or experienced COVID-19 illness. Likewise, Stafford et al.[18] conducted a prospective study among 37 mothers and 25 infants and found sIgA and sIgG in breast milk up to 6 months following maternal COVID-19 vaccination. In addition, they detected SARS-CoV-2 IgA and IgG in the stool of breastfeeding infants. These antibodies demonstrated the ability to neutralize the pseudovirus *in vitro*. Similarly, Longueira et al.[19] identified the presence of sIgA and sIgG against SARS-CoV-2 in human milk following maternal completion of two doses of the COVID-19 vaccination, with a minimum duration of 120 days. Moreover, the levels of Igs in breast milk exhibited a strong correlation with those found in the serum. These findings emphasize the potential for breastfeeding to serve as a crucial pathway for the transfer of protective immunity against SARS-CoV-2 from vaccinated or previously infected mothers to their infants.

Furthermore, our study's findings indicate that lactating mothers with hybrid immunity exhibited higher levels of sIgA, sIgG, and NAbs in their breast milk compared with mothers who received vaccination alone. These findings align with recent studies suggesting that hybrid immunity, resulting from both natural infection and vaccination, may contribute to the elevation of immune factors in breast milk. For instance, Golan et al.[20] observed that lactating mothers, who had encountered breakthrough SARS-CoV-2 infection post their third dose of immunization, displayed significantly higher sIgA in their milk following infection compared with those after their second and third vaccine doses. However, sIgG levels in their milk postinfection were found to be comparable with those observed after the third vaccine dose. There are several possible explanations for this result. First, most individuals with hybrid immunity had been exposed to SARS-CoV-2 antigens more repeatedly than individuals who were only immunized or infected. Moreover, there are differences in the quality of immune responses since the body is exposed to various antigens during infection, whereas mRNA and virus-vectored vaccines express only spike protein antigens. In addition, the infection with the recent variant SARS-CoV-2 and the vaccination may stimulate and expand the body's immune response.[21,22] This suggests a potential synergistic effect between vaccination and infection in enhancing the mucosal immune response in lactating mothers. Despite this, we observed the highest levels of NAbs in mothers who experienced SARS-CoV-2 infection without vaccination. However, caution is warranted in interpretation because of the small sample size of mothers with a history of SARS-CoV-2 infection alone, potentially impacting statistical power.

Although higher levels of breast milk immunities were observed when mothers received two doses of the COVID-19 vaccine alongside an infection compared with those who received vaccination alone, these differences disappeared when mothers received more than two doses of the immunization. One aspect to consider is whether the immune system reaches a saturation point, where additional doses do not result in further improvement in antibody levels. This could have implications for vaccination strategies, as administering more doses might not necessarily lead to a proportional increase in breast milk antibody levels. Nevertheless, to the best of our knowledge, this study is the first to compare immune responses in breast milk based on vaccination dosage, distinguishing between lactating mothers with hybrid immunity and those receiving vaccination alone. Therefore, for a better understanding of our findings, further research is indispensable. The dynamics of immune response in the context of breastfeeding are complex. Grasping these nuances is crucial for refining vaccination strategies and ensuring optimal protection for both mothers and infants against SARS-CoV-2.

Similarly, this study found that sIgA, sIgG, and NAbs in breast milk at 1 and 3 months showed no differences among lactating mothers with hybrid immunity, whether they had

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Table 4. Comparison of sIgA, sIgG, and NAbs in Human Milk Based on the Number of Vaccine Doses Between Lactating Mothers Who Had a History of SARS-CoV-2 Infection Together with Vaccination and COVID-Naïve Mothers Who Received Only the COVID-19 Immunization[a]

	sIgA ratio[a,b]			*sIgG ratio*[a,b]			*NAbs (%IH)*[a,b]		
Period of breast milk collection after infection/ vaccination	*COVID-19 vaccination in conjunction with SARS-CoV-2 infection*	*COVID-19 vaccination only*	*GMR (95% CI)*	*COVID-19 vaccination in conjunction with SARS-CoV-2 infection*	*COVID-19 vaccination only*	*GMR (95% CI)*	*COVID-19 vaccination in conjunction with SARS-CoV-2 infection*	*COVID-19 vaccination only*	*GMR (95% CI)*
1 month									
2 doses	3.64 (2.08–6.37)	0.7 (0.13–3.65)	5.23 (1.6–17.07)	1.74 (1.03–2.93)	1.78 (0.66–4.78)	0.98 (0.36–2.62)	85.9 (83.4–88.5)	85.4 (83.4–87.4)	1.01 (0.95–1.06)
3 doses	2.23 (1.12–4.45)	1.56 (0.7–3.47)	1.43 (0.43–4.76)	1.97 (1.27–3.05)	1.11 (0.22–5.61)	1.77 (0.64–4.87)	86.4 (82–91)	85.3 (81.6–89.2)	1.01 (0.93–1.11)
>3 doses	1.38 (0.56–3.39)	1.05 (0.04–28.24)	1.32 (0.15–11.44)	1.31 (0.85–2)	2.73 (0.01–10.0)	0.48 (0.16–1.41)	83.3 (80.5–86.3)	85 (48–152.4)	0.98 (0.9–1.07)
3 months									
2 doses	3.13 (1.86–5.27)	1.88 (0.13–27.2)	1.67 (0.44–6.38)	1 (0.66–1.53)	1.12 (0.89–1.39)	0.9 (0.37–2.21)	87.9 (85–90.9)	82 (74–90.9)	1.07 (0.99–1.16)
3 doses	2.77 (1.5–5.11)	1.68 (0.83–3.42)	1.65 (0.66–4.08)	1.08 (0.67–1.77)	1.4 (0.88–2.24)	0.77 (0.39–1.52)	87.3 (83.5–91.2)	79.9 (71.5–89.2)	1.09 (0.99–1.21)
>3 doses	2.08 (0.95–4.57)	0.67 (0.31–1.46)	3.12 (0.83–11.76)	1.29 (0.89–1.86)	1.2 (0.21–6.86)	1.07 (0.44–2.64)	86.9 (82.9–91.3)	85.2 (81.1–89.5)	0.99 (0.92–1.06)
6 months									
2 doses	3.47 (2.17–5.55)	1.16 (0.39–3.47)	2.99 (1.16–7.66)	1.22 (0.77–1.93)	0.23 (0.11–0.45)	5.4 (2.36–12.38)	81.4 (74.1–89.3)	86.1 (84.7–87.5)	0.95 (0.81–1.1)
3 doses	1.56 (0.82–2.99)	1.72 (0.67–4.41)	0.91 (0.3–2.71)	0.66 (0.44–0.98)	1.03 (0.52–2.02)	0.64 (0.31–1.3)	89.2 (87.3–91.2)	86.1 (83.5–88.9)	1.04 (1–1.07)
>3 doses	1.37 (0.7–2.67)	1.21 (0–375.6)	1.13 (0.23–5.45)	1.31 (0.64–2.71)	1.27 (0.01–10.6)	1.03 (0.19–5.58)	87.3 (82.3–92.7)	89 (75–106.2)	0.98 (0.86–1.12)

[a]Values are presented as GM (95% CI) and GMR (95% CI) using COVID-naïve as the reference group. The differences between the groups were performed by independent *t*-tests.

[b]sIgA and sIgG are expressed as a ratio of the extinction of test sample and calibrator, whereas NAbs are presented as a percentage of inhibition.

CI, confidence interval; COVID-19, coronavirus disease 2019; %IH, inhibition percentage; GM, geometric mean; GMR, geometric mean ratio; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; sIgA, specific immunoglobulin A; sIgG, specific immunoglobulin G; NAbs, neutralizing antibodies.

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received two, three, or more than three doses. However, at 6 months, mothers with triple COVID-19 vaccination and prior infection showed slightly lower sIgA and sIgG levels than those with hybrid immunity, receiving two vaccine doses, while exhibiting slightly higher levels of NAbs. Previous studies also indicated that administering booster doses of COVID-19 vaccination after a prior infection may restrict the enhancement of immunity against SARS-CoV-2. For example, Kim et al.[23] explored the immune response to a fourth dose of COVID-19 vaccination in individuals with prior natural infection with the Omicron variant and found that the fourth dose received 2 months after Omicron infection did not enhance surrogate virus neutralization titer at 50% against SARS-CoV-2. Furthermore, a recent study from England indicated that COVID-19 booster immunization may elevate the risk of myocarditis and pericarditis, particularly after the administration of the second vaccine dose. However, a reduced risk was observed in individuals with a history of prior SARS-CoV-2 infection before receiving COVID-19 vaccination.[24] This finding, coupled with the absence of an augmented effect following a booster, does not imply a spike-directed immune mechanism. The diminishing differences of immunities after individuals receive a booster dose, especially among those who had completed the primary vaccine series alongside a prior SARS-CoV-2 infection, may be influenced by the complex interplay of immune mechanisms, potential desensitization, or saturation of immune pathways. Further research should explore the optimal dosing strategies of COVID-19 vaccination to optimize immune protection for both mothers and infants during lactation.

The strength of this study is its longitudinal examination of the impact of hybrid immunity, not only for sIgA but also for sIgG and NAbs in human milk. It further compares breast milk immunity between individuals with no prior exposure to COVID and those with a history of SARS-CoV-2 infection coupled with immunization. In addition, the study explored the influence of booster vaccine doses on breast milk immunity. Nevertheless, there are several limitations to our study. Primarily, our focus was solely on examining immunity in breast milk, and we did not investigate the history of COVID-19 infection in infants. This omission raises concerns about the assurance that the observed levels of immunity in breast milk effectively transfer and safeguard infants from COVID-19 illness. Moreover, the limited sample size of milk samples, particularly from individuals with a history of SARS-CoV-2 infection alone, might have compromised our ability to detect variations in immunity levels associated with infection versus immunization.

Conclusion

In addition to a persistent presence of sIgA and sIgG against SARS-CoV-2 in the breast milk of lactating mothers who had hybrid immunity along with those who had vaccination or infection alone, our study demonstrated the *in vitro* ability of these antibodies in breast milk to neutralize the virus for up to 6 months. These immunities can be passed to infants, providing a safeguard against SARS-CoV-2 infections. Health care professionals should confidently support the continuation of breastfeeding during and after COVID-19 vaccination as well as infection.

Acknowledgments

The authors would like to express their deep appreciation to Professor Chitsanu Pancharoen and the dedicated team at Chula Kids Club for their invaluable support and assistance. Gratitude is also extended to Dr. Sophie Gallier for editing the article in English and providing valuable scientific advice. Special thanks go to Ms. Siriluck Poonkatkij and Ms. Nathawan Khunsri for their administrative support and contributions to data collection. The authors would also like to acknowledge Ms. Jiratchaya Sophonphan of The HIV Netherlands Australia Thailand Research Collaboration (HIV-NAT) at the Thai Red Cross AIDS Research Center for her role in statistical analysis. Finally, heartfelt thanks are extended to all the children and their families who willingly participated in this research. The authors also thank Chula Data Management Center, Faculty of Medicine, Chulalongkorn University, especially Miss Ashaya Ingkasatien, for their support in data management.

Informed Consent Statement

Legal guardians of the infants participating in this study provided informed consent following a detailed explanation of all study procedures by the researcher.

Authors' Contributions

The study's conception and design were carried out by O.S. and S.C. Data collection involved O.S., E.M., D.M., R.T., and S.K. Data analysis was conducted by O.S., E.M., S.K., P.S., and N.H. O.S. and S.C. interpreted the data. O.S., N.H., and S.C. revised the article, and all authors jointly approved the final draft.

Author Disclosure Statement

No competing financial interests exist.

Funding Information

This study was funded by the Ratchadapiseksompotch Fund, Faculty of Medicine, Chulalongkorn University, grant number RA2565-019, and Dairy Goat Co-operative (NZ) Limited and the New Zealand Ministry for Primary Industries (as part of Caprine Innovations NZ Sustainable Food & Fiber Futures Partnership program).

Data Availability

Data described in the article, codebook, and analytic code will be made available upon request in a de-identified form.

Supplementary Material

Supplementary Table S1–S3

References

1. Mosca F, Giannì ML. Human milk: Composition and health benefits. Pediatr Med Chir 2017;39(2):155; doi: 10.4081/pmc.2017.155

2. Sánchez C, Franco L, Regal P, et al. Breast milk: A source of functional compounds with potential application in nutrition and therapy. Nutrients 2021;13(3):1026; doi: 10.3390/nu13031026
3. Duijts L, Jaddoe VW, Hofman A, et al. Prolonged and exclusive breastfeeding reduces the risk of infectious diseases in infancy. Pediatrics 2010;126(1):e18-25–e25; doi: 10.1542/peds.2008-3256
4. Ho NT, Li F, Lee-Sarwar KA, et al. Meta-analysis of effects of exclusive breastfeeding on infant gut microbiota across populations. Nat Commun 2018;9(1):4169; doi: 10.1038/s41467-018-06473-x
5. Kramer MS, Kakuma R. Optimal duration of exclusive breastfeeding. Cochrane Database Syst Rev 2012;2012(8): Cd003517; doi: 10.1002/14651858.CD003517.pub2
6. Meek JY, Noble L, Section on Breastfeeding. Policy statement: Breastfeeding and the use of human milk. Pediatrics 2022;150(1):e2022057988; doi: 10.1542/peds.2022-057988
7. WHO. COVID-19 Public Health Emergency of International Concern (PHEIC) Global research and innovation forum 2020. Available from: https://www.who.int/publications/m/item/covid-19-public-health-emergency-of-international-concern-(pheic)-global-research-and-innovation-forum December 4, 2023.
8. Latorre G, Martinelli D, Guida P, et al. Impact of COVID-19 pandemic lockdown on exclusive breastfeeding in non-infected mothers. Int Breastfeed J 2021;16(1):36; doi: 10.1186/s13006-021-00382-4
9. Piankusol C, Sirikul W, Ongprasert K, et al. Factors affecting breastfeeding practices under lockdown during the covid-19 pandemic in Thailand: A cross-sectional survey. Int J Environ Res Public Health 2021;18(16); doi: 10.3390/ijerph18168729
10. Juncker HG, Mulleners SJ, Ruhé EJM, et al. Comparing the human milk antibody response after vaccination with four COVID-19 vaccines: A prospective, longitudinal cohort study in the Netherlands. EClinicalMedicine 2022;47:101393; doi: 10.1016/j.eclinm.2022.101393
11. Juncker HG, Mulleners SJ, van Gils MJ, et al. The levels of SARS-CoV-2 specific antibodies in human milk following vaccination. J Hum Lact 2021;37(3):477–484; doi: 10.1177/08903344211027112
12. Juncker HG, Romijn M, Loth VN, et al. Antibodies against SARS-CoV-2 in human milk: Milk conversion rates in the Netherlands. J Hum Lact 2021;37(3):469–476; doi: 10.1177/08903344211018185
13. Ricciardi A, Zelini P, Cassaniti I, et al. Serum and breastmilk SARS-CoV-2 specific antibodies following BNT162b2 vaccine: Prolonged protection from SARS-CoV-2 in newborns and older children. Int J Infect Dis 2022;122:905–909; doi: 10.1016/j.ijid.2022.06.055
14. van Keulen BJ, Romijn M, Bondt A, et al. Human milk from previously COVID-19-infected mothers: The effect of pasteurization on specific antibodies and neutralization capacity. Nutrients 2021;13(5):1645; doi: 10.3390/nu13051645
15. Palmeira P, Carneiro-Sampaio M. Immunology of breast milk. Rev Assoc Med Bras (1992)2016;62(6):584–593; doi: 10.1590/1806-9282.62.06.584
16. Ware J, McElhinney K, Latham T, et al. Sustained and boosted antibody responses in breast milk after maternal SARS-CoV-2 vaccination. Breastfeed Med 2023;18(8): 612–620; doi: 10.1089/bfm.2023.0106
17. Suteerojntrakool O, Mekangkul E, Ananta P, et al. The persistence of specific immunoglobulin A against SARS-CoV-2 in human milk after maternal COVID-19 vaccination. Breastfeed Med 2023;18(12):943–950; doi: 10.1089/bfm.2023.0210
18. Stafford L, Valcarce V, Henry M, et al. Detection of SARS-CoV-2 IgA and IgG in human milk and breastfeeding infant stool 6 months after maternal COVID-19 vaccination. Res Sq 2022; doi: 10.21203/rs.3.rs-1950944/v1
19. Longueira Y, Ojeda DS, Battistelli RBA, et al. SARS-CoV-2-Specific IgG and IgA response in maternal blood and breastmilk of vaccinated naïve and convalescent lactating participants. Front Immunol 2022;13:909995; doi: 10.3389/fimmu.2022.909995
20. Golan Y, Ilala M, Gay C, et al. Milk antibody response after 3rd dose of COVID-19 mRNA vaccine and SARS-CoV-2 breakthrough infection and implications for infant protection. medRxiv 2022; doi: 10.1101/2022.12.12.22283367
21. The Lancet Infectious D. Why hybrid immunity is so triggering. Lancet Infect Dis 2022;22:1649; doi: 10.1016/s1473-3099(22)00746-0
22. Sette A, Crotty S. Immunological memory to SARS-CoV-2 infection and COVID-19 vaccines. Immunol Rev 2022; 310(1):27–46; doi: 10.1111/imr.13089
23. Kim J, Seo H, Kim HW, et al. Effect of previous COVID-19 vaccination on humoral immunity 3 months after SARS-CoV-2 omicron infection and booster effect of a fourth COVID-19 vaccination 2 months after SARS-CoV-2 omicron infection. Viruses 2022;14(11); doi: 10.3390/v14112458
24. Stowe J, Miller E, Andrews N, et al. Risk of myocarditis and pericarditis after a COVID-19 mRNA vaccine booster and after COVID-19 in those with and without prior SARS-CoV-2 infection: A self-controlled case series analysis in England. PLoS Med 2023;20(6):e1004245; doi: 10.1371/journal.pmed.1004245

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