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Short Paper

SARS-CoV-2 antibodies in breast milk of women given one and two doses of COVID-19 vaccine

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***Background & objectives*: The COVID-19 pandemic underscores the significance of vaccination in mitigating disease spread, with Covishield and Covaxin serving as pivotal vaccines in India. Breast milk, rich in vital antibodies like IgA and IgG, plays a crucial role in enhancing the immune defence of breastfeeding infants. However, limited research exists on the antibody responses in breast milk among individuals receiving single versus double doses of the COVID-19 vaccine. This study aimed to bridge this gap by exploring IgA and IgG antibody levels in breast milk and assessing the correlation with COVID-19 vaccination status.**

***Methods*: This hospital-based descriptive study aimed to assess the relationship between COVID-19 vaccination and the presence of anti-SARS-CoV-2 IgA/IgG antibodies in breast milk. Breast milk samples were collected using a sterile, closed-system electric breast pump and stored at -20°C. ELISA testing, utilizing commercially available kits, was utilized to assess anti-SARS-CoV-2 IgA and IgG antibodies.**

***Results*: Among the 151 women participants, 76 (50.3%) received COVID-19 vaccination. Of these vaccinated women, 70 (92.1%) received Covishield, and 6 (7.9%) received Covaxin. Within the vaccinated cohort, 32 (42.1%) completed the recommended double-dose regimen, while 44 (57.9%) received a single dose. While no significant association was found between vaccination status and IgA positivity (P=0.491), a notable association emerged for IgG positivity (P<0.001). Notably, individuals who completed the recommended double-dose regimen exhibited higher IgA (63.6%) and IgG (65.4%) positivity compared to those receiving a single dose.**

***Interpretation & conclusions*: This study underscores the significance of COVID-19 vaccination in impacting IgA and IgG antibody presence in breast milk. Completing the double-dose regimen correlated with higher IgA and IgG levels, emphasizing the benefits of complete vaccination. These findings contribute to understanding vaccination's impact on maternal-infant health.**

Key words Antibodies - breast milk - ELISA - IgA - IgG - immunity - SARS-CoV-2

The COVID-19 pandemic caused by SARS-CoV-2 has posed unprecedented global health challenges. Vaccination is a pivotal strategy in curbing disease transmission and severity[1]. In India, Covishield and

Covaxin are frontline vaccines endorsed for adults aged ≥18 yr, including pregnant and breastfeeding women, healthcare workers, older adults, and the immunocompromised. Rigorous clinical trials and regulatory approvals affirm their safety and efficacy. Notably, no COVID-19 vaccines are authorized or approved specifically for infants[2].

Human milk contains crucial immunoglobulins (Igs) such as IgA, secretory IgA (SIgA), IgM, secretory IgM (SIgM), and IgG, which play vital roles in immune protection. IgA, predominant in human milk, defends the infant's gastrointestinal and respiratory tracts by preventing pathogen attachment[3,4]. IgG, transferred from the mother's bloodstream to breast milk, provides systemic immune protection, offering infants passive immunity. Lactating individuals infected with SARS-CoV-2 transmit specific antibodies via breast milk, enhancing infants' passive immunity. Vaccination of lactating individuals also transfers vaccine-induced antibodies to infants through breast milk, bolstering their immune defense[5,6].

Limited research has addressed the comparison of anti-SARS-CoV-2 antibody responses in breast milk between individuals who received a single dose of the vaccine and those completing the double-dose regimen. This study aims to fill this gap by evaluating anti-SARS-CoV-2 IgA and IgG levels in breast milk and exploring how COVID-19 vaccination correlates with antibody presence. Specifically, it compares IgA and IgG levels in breast milk between single-dose and double-dose vaccinated groups, shedding light on the impact of vaccination schedules on passive immunity in breastfeeding infants.

Materials & Methods

This cross-sectional study employed a hospital-based investigation approach to examine the association between COVID-19 vaccination and the presence of anti-SARS-CoV-2 IgA/IgG antibodies in breast milk.

Ethical approval was obtained from the Institutional Ethics Committee [II-PGTSC-IIA/P4]. Written informed consent was obtained from all participants before their inclusion in the study. Breast milk samples were collected from 151 healthy women donors registered in a Regional Human Milk Bank from October 2021 to December 2022. After obtaining written informed consent from the participants, a thorough physical examination of the nipple including areolar region was conducted to ensure the absence of any wounds or lesions. Additionally, serological tests for HIV, Hepatitis B and C, and syphilis were performed as part of the screening process to ensure donor safety. A closed-system electric breast pump was used for milk expression. Trained staff members collected the breast milk samples following strict hygienic precautions.

Two to four millilitres of donor human milk was collected and transported to the Department of Microbiology for further processing. The samples were transferred into sterile containers and labelled with a unique identifier. During transportation, the samples were placed in a dedicated transport box with cold packs. The samples were subjected to centrifuge at 1000 x g for 10 min. The supernatant containing the target antibodies was carefully collected using sterile pipette. The aliquoted breast milk samples were stored in cryovials at a temperature of -20°C to preserve the stability and integrity of the antibodies until further analysis[4,5].

Commercially available ELISA kits were used to determine the levels of anti-SARS-CoV-2 IgA and IgG antibodies in donor human milk. The levels of anti-SARS-CoV-2 IgA antibodies in donor human milk were determined using the Nova Lisa SARS-CoV-2 (COVID-19) IgA ELISA kit, (Nova Tech Immuno Diagnostica GmbH, Berlin, Germany). Microtiter plates coated with SARS-CoV-2 nucleocapsid antigens were used. After washing to remove unbound material, a horseradish peroxidase (HRP) labelled to human IgA conjugate were used to capture antibodies. Following further washing, tetramethylbenzidine (TMB) substrate was added, producing a blue reaction product. Absorbance at 450/620 nm was read using an ELISA microplate reader. The interpretation involved comparing sample absorbance to the cut-off value, yielding results in NovaTec Units (NTU), where >11 NTU indicates positive, 9-11 NTU equivocal, and <9 NTU negative.

The levels of anti-SARS-CoV-2 IgG antibodies in donor human milk were determined using the Q-LISA COVID Coronavirus IgG (COVID-19) ELISA Antibody Test (QED Bioscience Inc., San Diego, CA, United States). The nucleocapsid protein (N-protein) of SARS-CoV-2, pre-coated on microtitre plate wells, binds with COVID-19 IgG in the sample. This complex is captured by anti-human IgG-HRP, producing a colour with TMB substrate. The assay is positive if test absorbance exceeds the cut-off value. Validity requires the positive control mean (PCx) >0.5 and negative

Table: Association of COVID vaccination status with IgA and IgG antibody positivity amongst human milk donors

Antibody	Total participants (n=151)	Not vaccinated (n=75)		Vaccinated (n=76)		Statistical significance
		n	%	n	%	
IgA positive	40	18	24	22	28.9	χ^2=0.474; *P*=0.491
IgG positive	30	4	5.3	26	34.2	χ^2=19.772; *P*<0.001
COVID vaccination	Total (n=76)	IgA negative	IgA positive	IgG negative	IgG positive	
Single dose	44	36 (66.7)	8 (36.4)	35(70)	9 (34.6)	χ^2=5.888; *P*= 0.015
Double dose	32	18 (33.3)	14 (63.6)	15(30)	17 (65.4)	χ^2=8.786; *P*= 0.003

control mean (NCx) <0.2. The sample-to-cut-off ratio determines antibody presence.

Descriptive statistics were employed to summarize the demographic characteristics of the study participants. Categorical variables were presented as frequencies and percentages, while continuous variables were expressed as means with standard deviations. The Chi-square test was used to assess the associations between COVID vaccination status and the presence of IgA/IgG antibodies in breast milk. A *P*-value of <0.05 was considered statistically significant for all statistical analyses. Statistical analyses were conducted using SPSS software version 27 (IBM Corp., Armonk, NY, USA). Assumptions for the Chi-square test included the sample data randomly selected from the population, the variables were categorical, the expected frequency for each category was at least 5, observations were independent of each other, and no adjustments were made for multiple comparisons in this analysis. The statistical tests were two-tailed, and confidence intervals (CI) were set at 95 per cent.

Results

The average age of milk donors was 27.7 yr (SD±4.4 yr), ranging from 19 to 40 yr. Among them, 42.4 per cent were under 25 yr, 52.3 per cent were between 25 and 35 yr, and 5.3 per cent were 35 yr or older. Only 50.3 per cent (76/151) of the donors were vaccinated against COVID-19, with 92.1 per cent receiving Covishield and 7.9 per cent receiving Covaxin. Of the vaccinated donors, 42.1 per cent had completed the full two-dose regimen, while 57.9 per cent had received only one dose.

In the study population, 18 per cent tested positive for IgA and 19.9 per cent for IgG. Among unvaccinated donors, 28.9 per cent tested positive for IgA and 5.3 per cent for IgG. Among vaccinated donors, 24 per cent tested positive for IgA and 34.2 per cent for IgG, with a significant association between vaccination and IgG positivity (*P*<0.001), but not IgA positivity (*P*=0.491).

Further, within the vaccinated group, those who received two doses had higher positivity rates for both IgA (63.6%) and IgG (65.4%) compared to those who received only one dose (IgA: 36.4%, IgG: 34.6%), indicating a significant association between the number of vaccine doses and antibody presence (*P*<0.05 for both) (Table).

Breast milk, a vital source of nutrition for infants, also supports their immune system development[7]. Given the ongoing COVID-19 pandemic, it is crucial to understand the presence of anti-SARS-CoV-2 antibodies in breast milk and their potential protective role for infants. Assessing the impact of COVID-19 vaccination on antibody transfer through breast milk among lactating women provides valuable insights into maternal and infant health.

In our study, 50.3 per cent of the 151 participants received COVID-19 vaccinations, which was lower than the rates in previous studies that reported vaccination rates ranging from 70 to 90 per cent among lactating individuals[8,9]. Importantly, 42.1 per cent completed the recommended vaccine schedule with both doses. Factors like the study population, location, vaccine availability, and hesitancy probably influenced these variations.

In our study of 151 breast milk donors, 26.5 per cent tested positive for IgA antibodies and 19.9 per cent for IgG antibodies. Previous studies reported IgA positivity rates from 1.7 to 30 per cent and IgG rates from 0 to 28 per cent[10-12]. These variations reflect factors such as local SARS-CoV-2 prevalence, timing of testing relative to infection or vaccination, and test characteristics. Assessing SARS-CoV-2 antibodies in breast milk underscores the role of transference of passive immunity through breastfeeding during

the pandemic, adding crucial insights to existing literature[13].

In our study, vaccinated milk donors showed a higher percentage of IgG-positive individuals than non-vaccinated donors, consistent with previous research[14] highlighting increased IgG post-vaccination. IgG antibodies are crucial in protecting against SARS-CoV-2 infection. However, we found no significant association between COVID-19 vaccination and IgA positivity among donors aligning with prior studies[15]. Vaccination timing, vaccine type, and individual immune responses influence antibody levels and detection.

In our study, individuals who received a single dose of the COVID-19 vaccine showed lower IgA and IgG positivity compared to those who received both doses. This trend was consistent across both antibody types. Receiving both doses of the COVID-19 vaccine appears to elicit a more robust immune response in the Indian population. A previous study found that after the first vaccine dose, 64 per cent of breast milk samples were positive for IgA and 30 per cent for IgG. After the second dose, these rates increased to 70 per cent for IgA and 91 per cent for IgG[16]. However, some studies suggested limited transfer of vaccine-generated antibodies through breastfeeding[17].

The similarity in IgA antibody response between vaccinated and non-vaccinated individuals might be influenced by natural virus exposure, stimulating IgA production independent of vaccination. Other factors, such as environmental or genetic influences, could also affect IgA levels. Conversely, the significant difference in IgG antibody response likely resulted from COVID-19 vaccines' targeted immunogenicity, designed to stimulate a robust IgG response crucial for systemic immunity. Vaccination appears pivotal in enhancing IgG production, while individual immune responses and pre-existing immunity might also contribute to variations in the antibody levels. Passive immunity via breast milk transfers maternal IgA and IgG antibodies to infant mucosal surfaces, offering localized protection, especially in the gastrointestinal tract. Despite potential degradation, intact antibodies provide protective effects in distant tissues. While effective against SARS-CoV-2 in mucosal surfaces like the mouth and intestines, their role in preventing respiratory infections is uncertain. Nevertheless, this local protection can indirectly enhance overall immune defence by reducing viral load and maintaining robust mucosal immunity, which is critical for infant health and resilience against pathogens[18].

Highly effective COVID-19 vaccines have been crucial in controlling virus spread, yet newborns lack immediate vaccination access due to safety concerns. Breast milk offers newborns COVID-specific antibodies as a preventive measure. Our study contributes to the limited literature on IgA and IgG antibodies against SARS-CoV-2 in Indian donor breast milk. Donors receiving two vaccine doses showed higher antibody levels, suggesting vaccination enhances breast milk antibody response, potentially protecting breastfed newborns from infection and severe COVID-19. It is essential to recognise the limitations of our study, including the lack of data on prior SARS-CoV-2 infections and the potential confounding effects of infection-induced antibodies. Future research should aim to address these gaps and further elucidate the role of breast milk antibodies in infant health and immunity.

Our study demonstrated that breast milk from donors who received two doses of COVID-19 vaccines had higher levels of IgA and IgG antibodies than those who received a single dose. This finding underscores the importance of completing the full vaccination regimen to enhance the antibody response in breast milk, potentially offering better protection to breastfeeding infants against SARS-CoV-2. However, we also noted no significant association between vaccination status and IgA positivity, suggesting that natural exposure to the virus may play a role in IgA production. These findings highlight the complex interplay between vaccination and natural infection in shaping immune responses.

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