1. Records of antibodies in breast milk in postpartum women who have been vaccinated or exposed

by Eighty Mardiyan Kurniawati

SYSTEMATIC REVIEW

REVISED **Records of antibodies in breast milk in postpartum women who have been vaccinated or exposed to COVID-19: A systematic review [version 3; peer review: 1 approved]**

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Abstract
Background: Breast milk is a critical element in developing a baby's immunity through immune transfer. Antibodies are an essential unit of immunity against infection with the SARS-CoV-2 virus. This paper explores antibodies in breast milk in postpartum women who have been vaccinated or exposed to coronavirus disease 2019 (COVID-19). Duration of antibody appearance was studied to determine the adequate time in transferring antibodies by breastfeeding.
Methods: Three databases, PubMed, Google Scholar, and ScienceDirect, were used as sources of articles. Inclusion criteria applied in selecting articles were prospective observational study or experimental design study in English, evaluating antibodies in breast milk, and conducted between 2019–2021. Article quality and risk of bias were assessed with Critical Appraisal Skills Programme (CASP). The data found were synthesized in a narrative manner.
Results: This systematic review included 20 articles. A total of 306 postpartum women who were infected with COVID-19, 20 postpartum women who had viral symptoms and 495 postpartum women who had been vaccinated were studied. Immunoglobulin A (IgA) and immunoglobulin G (IgG) antibodies were found in the breast milk of infected and vaccinated postpartum women. SARS CoV-2 infection is associated with the presence of IgA dominant, whereas vaccination is related to the presence of IgG dominant. Antibodies persisted from day 10 of onset to 10 months in infected postpartum women and started from three days to six weeks in vaccinated postpartum women. Meta-analysis could not be carried out due to the variety of articles.
Conclusions: Antibodies found in breast milk in infected and vaccinated postpartum women have different dominant types. Further research needs to be done regarding the mechanism of antibody

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Any reports and responses or comments on the article can be found at the end of the article.

transfer in breast milk, longer research duration and studies that directly examine the comparison of antibodies in breast milk in vaccinated and infected postpartum women.

Registration: PROSPERO (CRD42022340859, 23 June 2022).

Keywords

COVID-19, maternal health, neonatal health, human milk, antibody, vaccine

This article is included in the Emerging Diseases and Outbreaks gateway.

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REVISED **Amendments from Version 2**

Due to comments from reviewers, we as authors have changed the version. We add a description of the sample type in "comparison of antibody levels by type of vaccine" section.

Any further responses from the reviewers can be found at the end of the article

Introduction

SARS CoV-2 became a worldwide pandemic and caused changes in all aspects of life including in mothers with postpartum and neonatal period.[1] Coronavirus disease 2019 (COVID-19) infection during pregnancy, birth and postpartum is associated with a significant increase in morbidity and mortality in mothers and babies. New mothers and their infants are more susceptible to infection compared to the general population.[2] COVID-19-related postpartum deaths have been reported in Brazil, with an estimated 106 deaths in 2020.[3] In addition, babies are also vulnerable to COVID-19 infection. The babies born to mothers infected with COVID-19 are more likely to be admitted to the neonatal unit.[4] Case overview of COVID-19 in children in previous studies were seen to be less frequent, less severe, and the mortality rate very low, but there is growing evidence that they are as susceptible as adults.[5] Infants with severe respiratory failure and the prolonged clinical course associated with SARS-CoV-2 exposure may be due to extreme prematurity, immature lungs, and immunocompromised status.[6]

Breast milk is the only food needed in the first 6 months of life.[7] Breastmilk also the best source protection for babies.[8] Breast milk contains various kinds of antibodies that provides protection to the baby. One way for babies to get additional antibody protection is through vaccination. In the case of COVID-19, vaccination is one of the main protections but the provision of a COVID-19 vaccine for newborns and babies are not yet available. A recent study found that a COVID-19 vaccination regimen consisting of BNT162b2 was found to be safe, immunogenic, and efficacious in children aged five to 11 years.[9] When compared with infants, postpartum mothers have the opportunity to get antibodies through vaccination. The COVID-19 vaccine is an effective way to prevent COVID-19.[10] In influenza outbreaks in previous years, influenza vaccines have been shown to increase serum antibodies and reduce disease severity in both mother and baby.

Breastfeeding can protect for at least six months because breast milk contains consistently high levels of actively produced anti-influenza immunoglobulin A (IgA). The results of this research found that breastfeeding can provide protection for at least the first 6 months because breast milk contains consistently high levels of actively produced anti-influenza immunoglobulin A (IgA). Infants with fever have fewer episodes of respiratory illness, which implies that breastfeeding may provide local mucosal protection.[11] The risk of COVID-19 can be reduced through breastfeeding among children as has been documented for other infections compared to formula feeding.[12]

The risk of being exposed to the virus and providing nutrition is a dilemma, especially regarding the content of substances contained in breast milk. Breastfeeding mothers do not understand the substances contained in breast milk, whether viruses or protective substances when giving it to their babies. This condition also exacerbated by the rise of misinformation on social media about the content of the SARS-CoV-2 virus in breast milk, causing breastfeeding issues during the pandemic.[13] Breastfeeding mothers will be distraught and ask themselves whether the coronavirus can be transmitted through breast milk and what they can do to protect themselves and their babies.[8] The World Health Organization (WHO) said breastfeeding does not need to be stopped during COVID-19 infection or after the mother's vaccination.[14] Research conducted by United Nations Children's Fund (UNICEF) in five countries in South Asia found that less than 25% of interviewees understood that it was safe to continue breastfeeding. They preferred to give formula milk,[15] whereas a meta-analysis conducted in 2020 showed that the SARS-CoV-2 genome is generally not found in the breast milk of breastfeeding mother infected with COVID-19.[16] After the virus content information has been clearly explained, the protection points become an essential part to know. Previous studies have found that there are antibodies in the breast milk of COVID-19-infected mothers, but the duration of the presence of these antibodies is unknown.[17] Infected and vaccinated postpartum women allow the formation of an antibody against COVID-19, but the long-term impact on antibody composition and functional activity is unclear.[18] Given this context, the purpose of this research is to determine the status of antibodies in breast milk following COVID-19 infection and vaccination. After finding records of the presence of antibodies, the duration of the appearance of antibodies will also be studied. Knowledge about the duration of antibodies in breast milk determines the adequate time in transferring antibodies by breastfeeding. The current results can be used as a guideline for recommendations to continue breastfeeding and health promotions that emphasize the presence of antibodies in breast milk in vaccinated postpartum mothers.

Methods

Study design and search strategy

The status of antibodies in breast milk after exposure to COVID-19 and after vaccination and the duration of antibodies appearing in breast milk were explored by compiling a systematic review. The preparation of this report follows the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines.[19,39] This systematic review is registered in PROSPERO (CRD42022340859, 23 June 2022). Data were collected in July-September 2021. English-language research conducted from 2020 to 2021 according to topics was searched in PubMed (RRID: SCR_004846), Google Scholar (RRID:SCR_008878), and Science Direct. The keywords used were a combination of Medical Subject Heading (MeSH) terms and relevant keywords in a different order: "breast milk", "COVID-19", "antibody", "immunoglobulin", "vaccine", "severe", "acute respiratory syndrome coronavirus 2", "coronavirus disease 2019", "SARS-CoV-2". The author also uses synonyms in the search. In the articles found, the researcher also examined the literature in the bibliography, including manuscripts that were not captured in the electronic literature search.

Study selection

The inclusion criteria applied in selecting articles were prospective observational study or experimental design study in English, evaluating antibodies in breast milk, and the study was conducted during the COVID-19 pandemic between 2019 and 2021. The exclusion criteria for this study were case reports, animal studies, letters to editors, study reviews, abstracts without full text. Manuscripts that only discussed breast milk in healthy postpartum or breastfeeding mothers were not included.

Data collection process

Two authors (EMK and NAR) performed title and/or abstract screening independently of the included articles using standard Microsoft Excel (RRID:SCR_016137) forms. The data obtained were combined in one folder and then an assessment was carried out. Each author analyzed all existing manuscripts, and then the results were compared with each other. A third external collaborator was consulted (Hari Paraton) to address disagreements in consensus.

Data items

The outcomes from this study were the presence of antibodies and the duration of the appearance of antibodies in breast milk. The antibodies studied were IgG and IgA against COVID-19. The tool used to detect the presence of antibodies is ELISA or similar tools. The authors realized that not all manuscripts had explanations for these two antibody levels, therefore the studies that only discussed one type of antibody were also included in the systematic review. The authors also realized that not all studies that discussed antibodies also explained the duration of the appearance. Based on the consideration of the limited number of articles, the authors discussed the articles that discusses the time when antibodies can be detected. Unclear information was included in the exclusion criteria.

Study risk of bias assessment

Authors conducted a risk of bias assessment study using critical appraisal tools. The Critical Appraisal Skills Programme (CASP) was used to assess the formal article by two independent research team members, EMK and NAR. The use of CASP is based on the 1994 JAMA 'Users' guides to the medical literature, which is used for both randomized controlled trials and systematic reviews. This checklist was adapted from Guyatt GH, Sackett DL, and Cook DJ and is used by health care practitioners.[20] CASP results are concluded into the category of moderate overall quality and low overall quality.[21] The results of the analysis are presented in Table 1.

Effect measures

Due to the limited number of manuscripts, a meta-analysis could not be completed. The effect that was measured in this study was the number of respondents' breast milk presentations where antibodies were found in breast milk. The obtained durations were also combined descriptively.

Data abstraction and synthesis

The study selection process was through a review of the inclusion and exclusion criteria. As suggested in a systematic review of the literature, the analysis in this study was based on the findings of each study. The steps started from extracting the relevant results, sorting, and examining them to identify sub-themes and themes. The type of synthesis used is in the form of narrative synthesis. Narrative synthesis was chosen because it allowed authors to gather insight into the antibody content in breast milk and we did not perform this meta-analysis. The data are arranged in Table 2 contains the characteristics of research on mothers infected with COVID-19 and antibody status in breast milk and is written in a systematic table including (1) authors, year, country, (2) research time, (3) study type, (4) COVID-19 confirmation, (5) control group, (6) sampling time, (7) number of infected mothers, (8) antibody test, (9) number of mothers showing antibodies, and (10) duration time. Table 3 contains characteristics of research on mothers with COVID-19 vaccination and antibody status in breast milk and is written in a systematic table including (1) authors, year, country, (2) research

Table 1. The results of the critical appraisal using CASP checklist.

Author	Did the study address a clearly focused issue?	Was the cohort recruited in an acceptable way?	Was the exposure accurately measured to minimize bias?	Was the outcome accurately measured to minimize bias?	Have the authors identified all important confounding factors?	Have the authors taken account of the confounding factors in the design and/or analysis?	Was the follow up of subjects complete enough?	Was the follow up of subjects long enough?	How precise are the results?	Do you believe the results?	Can the results be applied to the local population?	Do the results of this study fit with other available evidence?	What are the implications of this study for practice?	Overall quality assessment
Juncker et al.[22]	Y	Y	Y	Y	Y	Y	C	C	IgA $p < .001$ and IgG $p = .011$	Y	Y	Y	Y	M
Juncker et al.[40]	Y	Y	Y	Y	N	N	Y	Y	$p = .070$	Y	Y	Y	Y	M
Narayanaswamy et al.[41]	Y	Y	Y	Y	Y	Y	C	C	$p > 0.05$	Y	Y	Y	Y	M
Bäuerl et al.[42]	Y	Y	Y	Y	N	N	C	C	$p < 0.0001$	Y	Y	Y	Y	M
Duncombe et al.[43]	Y	Y	Y	Y	N	N	C	C	NA	Y	Y	Y	Y	M
Pace et al.[44]	Y	Y	Y	Y	N	N	C	C	NA	Y	Y	N	Y	M
Fox et al.[45]	Y	Y	Y	Y	N	N	Y	Y	IgA $p < 0.0001$, and IgG $p = 0.017$	Y	Y	N	Y	M
Demers-Mathieu et al.[46]	Y	Y	Y	Y	N	N	C	C	$p = 0.002$	Y	Y	Y	Y	M
Lechosa-Muñiz et al.[23]	Y	Y	Y	Y	Y	Y	C	C	$p < 0.001$, $p = 0.001$	Y	Y	Y	Y	M
Gray et al.[25]	Y	Y	Y	Y	Y	Y	Y	Y	$p < .0001$	Y	Y	Y	Y	M
Low et al.[47]	Y	Y	Y	Y	N	N	Y	Y	NA	Y	Y	Y	Y	M
Jakuszko et al.[48]	Y	Y	Y	Y	N	N	C	C	$p < 0.001$	Y	Y	Y	Y	M
Guida et al.[49]	Y	Y	Y	Y	N	N	C	C	$p = 0.145$	Y	Y	Y	Y	M
Ramirez et al.[50]	Y	Y	Y	Y	N	N	C	C	$p < .001$	Y	Y	Y	Y	M
Perl et al.[51]	Y	Y	Y	Y	N	N	C	C	IgA $p < .001$ and IgG $p = .004$	Y	Y	Y	Y	M
Golan et al.[30]	Y	Y	Y	Y	N	N	C	C	$p < 0.0001$	Y	Y	Y	Y	M
Valcarce et al.[52]	Y	Y	Y	Y	Y	Y	C	C	$p = 0.005$	Y	Y	Y	Y	M
Selma-Roy et al.[24]	Y	Y	Y	Y	Y	Y	C	C	$p < 0.0001$	Y	Y	Y	Y	M
Juncker et al.[53]	Y	Y	Y	Y	Y	Y	C	C	$p < .001$	Y	Y	Y	Y	M
Rosenberg-Friedman et al.[54]	Y	Y	Y	Y	Y	Y	C	C	$p < 0.0125$	Y	Y	Y	Y	M

Note: CASP = Critical Appraisal Skills Programme, Y = yes, C = can't tell, N = No, N/A = not applicable, M = moderate overall quality, L = low overall quality. Further information for the question "What were the results of this study?" has been presented in Table 2 and Table 3.

Table 2. Characteristics of research on breastfeeding mothers infected with COVID-19 and antibody status in breast milk

Authors, year, country	Research time	Study type	COVID-19 confirmation	Control group	Sampling time	No. of infected mothers	Antibody test	No. of mothers showing antibodies, n (%)			Time when antibodies can be detected
								IgA	IgG	IgM	
Juncker et al.,[22] 2021, Netherlands	October 2020 and February 2021	Prospective cohort	PCR	Healthy lactating mother	An average of eight weeks after getting a positive result	165	ELISA	98 (59)	No data available	No data available	10 months after COVID-19 confirmation
Juncker et al.,[40] 2021, Netherlands	May–August 2020	Longitudinal follow-up	PCR and symptoms	No control used in this research	After the clinical symptoms in 14-143 days	24	ELISA	24 (100)	No data available	No data available	Five months (143 days) after the onset of COVID-19 symptoms
Narayanaswamy et al.,[41] 2021, MMC Worchester, MA	March–September 2020	Prospective cohort	PCR	Bilateral colostrum sample pre-pandemic as control (n = 8)	48 hours of delivery	15	ELISA	11 (73)	11 (73)	5 (33)	No data available
Bäuerl et al.,[42] 2021, Spain	April–December 2020	Prospective multicenter longitudinal study	PCR	Whole milk pre-pandemic control (n = 13)	Before and after delivery <7 days and 15 days	60	ELISA	51 (72.9)	45 (64.3)	51 (72.9)	206 days after PCR confirmation
Duncombe et al.,[43] 2021, USA	No data available	Prospective longitudinal cohort study	PCR	PCR negative control (n = 3)	Three weeks to six months post-illness onset	2	ELISA	2 (100)	2 (100)	1 (50)	10 days to six months after symptom onset
Pace et al.,[44] 2021, USA	No data available	Prospective study	PCR	Milk sample pre-pandemic control (n = 10)	Average 12.0 ± 8.9 days after onset of symptoms	18	ELISA	14 (76)	15 (80)	No data available	No data available
Fox et al.,[45] 2020, USA	December 2019	Prospective study	PCR and symptoms	Milk sample pre-pandemic control (n = 10)	14–30 days after symptoms	15	ELISA	12 (80)	2 (13)	1 (7)	No data available
Demers-Mathieu et al.,[46] 2021, USA	No data available	Unpaired experimental design	PCR	20 with viral symptom control and 16 unexposed	16–84 days after infection	7	ELISA	Y, no data available	Y, no data available	Y, no data available	No data available

Note: COVID-19 = coronavirus disease 2019, IgA = immunoglobulin A, IgG = immunoglobulin G, IgM = immunoglobulin M.

Table 3. Characteristics of research on vaccinated breastfeeding mothers and antibody status in breast milk.

Authors	Year	Country	Research time	Study type and sample timing	Vaccine type	Number of vaccinated women and the dose given	Antibody test	Antibody type			Finding	Time when antibodies can be detected
								IgA	IgG	IgM		
Lechosa-Muñiz et al.[23]	2021	Spain	1 April 2021 to 30 April 2021	Observational study 30 days after the second dose of the vaccine and 30 days after the first dose.	BNT162b2 mRNA-1273 ChAdOx1-S	20 women with a single dose of ChAdOx1-S. 70 women with two doses of BNT162b2, 20 women with two doses of mRNA-1273.	ELISA	Yes	Yes	NA	Breastfeeding mothers offer their infants IgA and IgG isotype antibodies directed against SARS-CoV-2 protein S in breast milk.[30]	No data available
Gray et al.[25]	2021	No data available	17 December 2020, and 23 February 2021	Prospective cohort study two to six weeks after the second vaccine.	BNT162b2 Pfizer/ BioNTech or mRNA-1273 Moderna/ National Institutes of Health (NIH)[34]	31 women with two doses of RNA vaccine.	ELISA	Yes	Yes	Yes	The second vaccine dose increased IgG, but not IgA, in maternal blood and breast milk.[33]	No data available
Low et al.[47]	2021	Singapore	No data available	Longitudinal study. Five time points: pre-vaccination (T1), 1–3 days after dose one (T2), 7–10 days after dose 1 (T3), 3–7 days after dose two of COVID-19 mRNA vaccine (T4), and 4–6 weeks after dose two of COVID-19 mRNA vaccine (T5).	BNT162b2 (Pfizer-BioNTech)	14 lactating healthcare workers after the second dose	ELISA	Yes	Yes	NA	86% (12/14) of the individuals produced SARS-CoV-2-specific IgA, and 100% (14/14) individuals produced SARS-CoV-2-specific IgG in human milk.[41]	86% of individuals produce SARS-CoV-2-specific IgA, and 100% of individuals produce SARS-CoV-2-specific IgG in breast milk within 3-7 days after administration second dose of BNT162b2 vaccine and decreased over time. Significant IgG decreased to 4-6 weeks after the second dose.[41]

Table 3. *Continued*

Authors	Year	Country	Research time	Study type and sample timing	Vaccine type	Number of vaccinated women and the dose given	Antibody test	Antibody type			Finding	Time when antibodies can be detected
								IgA	IgG	IgM		
Jakuszko et al.[48]	2021	Poland	No data available	Prospective cohort Sampling after the first dose of vaccine, on day 8 ± 1, on day 22 ± 2 (immediately before the second dose), on day 29 ± 3 (which is also day 7 ± 3 after the second dose), and on day 43 ± 4 (which is also day 21 ± 4 after the second dose).	mRNA vaccine BNT162b2 (Pfizer-BioNTech)	32 breastfeeding women.	ELISA	Yes	Yes	NA	There are IgG and IgA in breast milk. IgG is detectable in serum levels.	Serum and breast milk antibody concentrations were highest on day 29 ± 3 and decreased on day 43 ± 4. The immune response to vaccination was most vital 7 ± 3 days after the second dose of vaccine. Strong secretion of IgA and IgG antibodies in breast milk for six weeks after vaccination.
Guida et al.[49]	2021	Italy	No data available	Cohort prospective observational study, 17 breastfeeding women and 10 volunteers 20 days after the first dose and seven volunteers seven days after the second dose.	mRNA BNT162b2 vaccine	10 women after the first dose, seven women after the second dose.	ELISA	Yes	Yes	No	Anti-SARS CoV-2 S-IgA, IgG, and IgM antibodies in the breast milk of vaccinated women.	7 days after the second dose.
Ramirez et al.[50]	2021	No data available	22 February to 4 April 2021	Prospective cohort, 14 days after their second dose of vaccine.	94%, BNT162b2 mRNA COVID-19 vaccine and 6% COVID-19 mRNA-1273 vaccine	98 vaccinated, and the 24 control participants.	The SARS-CoV-2 IgG Architect Abbott®	Yes	Yes	NA	All participants had IgG antibodies, and 89% of them had IgA antibodies against SARS-CoV-2 in breast milk.	No data available

Table 3. *Continued*

Authors	Year	Country	Research time	Study type and sample timing	Vaccine type	Number of vaccinated women and the dose given	Antibody test	Antibody type IgA	Antibody type IgG	Antibody type IgM	Finding	Time when antibodies can be detected
Perl et al.[51]	2021	Israel	December 2020 and January 2021	Prospective cohort study before administration of the vaccine and then once weekly for six weeks starting at week two after the first dose.	All participants received two doses of the Pfizer-BioNTech	44 breastfeeding women after the first dose.	ELISA	Yes	Yes	NA	Strong neutralizing effects, protective effect against infection in the infant due to SARS-CoV-2.	SARS-CoV-2-specific IgA and IgG antibodies in breast milk six weeks after vaccination. IgA secretion was early, followed by a spike in IgG after four weeks (a week after the second vaccine). [78]
Golan et al.[30]	2021	USA	December 2020 to June 2021	Prospective cohort, 50 breastfeeding women collected from a subset of infants whose mothers received the vaccine during lactation or pregnancy (4–15 weeks after mothers' second dose).	mRNA46-based vaccines for COVID-19 (mRNA-1273 and BNT162b2) [87]	50 breastfeeding mothers after second dose.	ELISA	Yes	Yes	Yes	Anti-SARS-CoV-2 IgG and IgM levels significantly increased in maternal plasma, and significant transfer of anti-SARS-CoV-2 IgA and IgG antibodies to milk. Anti-SARS-CoV-2 IgG antibodies were not detected in the plasma of infants whose mothers were vaccinated during lactation. [85]	No data available
Valcarce et al.[52]	2021	USA	December 2020 to March 2021	Prospective observational study, 22 lactating health care workers. There were three time points (pre-vaccination, post-first vaccine, and post-second vaccine doses).	SARS-CoV-2 mRNA vaccine (Pfizer-BioNTech or Moderna)	20 lactating health care mothers.	ELISA	Yes	Yes	Yes	Significant secretion of SARS-CoV-2-specific IgA and IgG in human milk and plasma is discovered after SARS-CoV-2 vaccination. [54]	No data available

Table 3. *Continued*

Authors	Year	Country	Research time	Study type and sample timing	Vaccine type	Number of vaccinated women and the dose given	Antibody test	Antibody type			Finding	Time when antibodies can be detected
								IgA	IgG	IgM		
Selma-Royo et al.[24]	2021	Spain	January to April 2021	Prospective cohort observational study, 75 lactating women. Seven time points were collected from baseline up to 25 days after the 1st dose, and same points were collected for mRNA vaccines 30 days after second dose.	mRNA vaccines (BNT162b2 and mRNA-1273) and adenovirus-vectored vaccine (ChAdOx1 nCoV-19)	75 lactating women.	ELISA	Yes	Yes	NA	High intervariability for IgA antibodies. IgG levels were significantly higher than those observed in milk from women who had COVID-19, while IgA levels were lower.	No data available
Juncker et al.[53]	2021	Netherlands	2021	Prospective longitudinal study, 16 samples of human milk were collected according to a schedule: one sample before the first vaccination and one sample on days 3, 5, 7, 9, 11, 13, and 15–17 days after the first vaccination. This schedule was the same for the second vaccination.	mRNA based BNT162b2 vaccine	20 women after the second dose, six women after the first dose.	ELISA	Yes	Yes	NA	A SARS-CoV-2-specific antibody IgA response was observed in human milk.	IgA starting to increase between days 5–7 after the first dose and declining after day 15, on average.
Rosenberg-Friedman et al.[54]	2021	No data available	2021	Prospective cohort study approximately five months postpartum in the first dose (mean 154 days, range 68–382) and the second dose 21 days later.	BNT162b2 mRNA vaccination	10 lactating health care providers in first dose and second dose.	ELISA	Yes	Yes	NA	IgG and IgA with neutralization capacity.	14 days after the second dose.

Note: COVID-19 = coronavirus disease 2019, IgA = immunoglobulin A, IgG = immunoglobulin G, IgM = immunoglobulin M.

time, (3) study type and sample timing, (4) vaccine type, (5) number of vaccinated women and the dose given, (6) antibody test, (7) type of antibody, (8) finding, and (9) duration time. Heterogeneity in the data was explored including duration of baseline antibody measurement and sample characteristics.

Reporting bias assessment
The researcher was unable to form the funnel plot due to the limited number of the manuscript. Funnel plots have a similar function to forest plots. This plot serves to assess the intervention effect of individual studies on some measure or precision of each study. The risk assessment of bias in this review was carried out using a risk approach that emerged and was reported as a research limitation.

Certainty assessment
The main domain used to assess the certainty of evidence was the risk of bias through critical appraisal and the inconsistency of the results of the research included in the review.

Results
Results of article screening
In searching the database, 1,465 abstracts were found from searches with relevant keywords. The authors then screened results for possible inclusion. The findings of 935 articles were excluded from the eligibility list. The articles did not meet the criteria, including the type of manuscript, the language used, and the topics discussed under the proposed title. After this stage, the researcher tried to re-examine the assessment results, but the manuscript finally entered the exclusion criteria. After screening, 30 full-text articles were selected and examined in detail to determine eligibility. Furthermore, 20 articles were determined that met the requirements. There were eight studies on the antibody status of infected breastfeeding mothers and 12 studies on antibody status of vaccinated breastfeeding mothers included. Figure 1 shows the study selection flowchart. The quality of the research is in the moderate category and is arranged in Table 1.

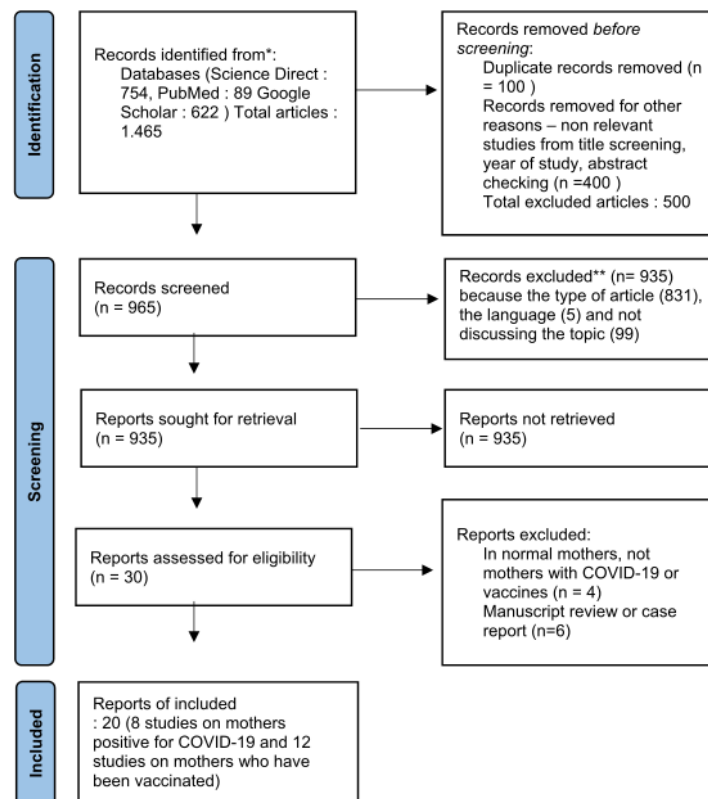**Figure 1. Flowchart study selection.** COVID-19, coronavirus disease 2019.

Antibody status in breast milk from infected breastfeeding mother

A total of 306 breastfeeding mothers who were positive for COVID-19 and 20 of them who had viral symptoms and had a very high likelihood that they had been exposed to COVID-19 were studied to determine antibody status in breast milk. Samples were taken within 48 hours after delivery to six months after infection. Table 2 describes characteristics of research on breastfeeding mothers infected with COVID-19 and antibody status in breast milk. The samples included in this review are breastfeeding mothers with positive PCR confirmation and mothers who had a very high likelihood that they had been exposed to COVID-19 when viewed from the symptoms they had. Almost all studies used controls, namely comparisons with healthy breastfeeding mothers and breastmilk samples taken during the pre-pandemic period. One study did not include controls, namely a longitudinal study. Detection of all antibodies was performed using ELISA. Most studies found that breast milk contains immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM). IgA was the dominant antibody found, while IgG and IgM were not always found in individuals. However, infected breastfeeding mothers did not always have IgA antibodies against COVID-19, which was found in a study by Juncker et al. (2021), who found that 59% of mothers had these antibodies.[22]

Antibody status in breast milk from vaccinated breastfeeding mother

The characteristics of research on vaccinated breastfeeding mothers and antibody status in breast milk can be seen in Table 3. A total of 495 breastfeeding mothers who had been vaccinated, received the first and second doses of various types of vaccines and had never had COVID-19 were analyzed. IgG and IgA antibodies were found in the breast milk of vaccinated mothers. No studies showed IgM data. Administration of the second dose of the vaccine was associated with an increase in IgG in breast milk. Despite evidence of increased antibodies, anti-SARS-CoV-2 IgG antibodies were not detected in the plasma of infants whose mothers were vaccinated during breastfeeding.[30] This study is one of 20 studies examining infant plasma. In the study, infant blood was collected with a heel stick at 5-15 weeks after breastfeeding mothers were vaccinated the second dose. Eight blood samples were collected from infants aged 68 days to 1 year. IgG levels were higher than IgA levels in the breast milk of vaccinated breastfeeding mothers.

Time when antibodies can be detected

In mothers infected with COVID-19, the appearance of antibodies was detected on day 10 and could persist for up to 10 months. IgA levels are known to decrease over time, while IgG is relatively stable. In vaccinated mothers, antibody status differed between mothers vaccinated with first dose and second dose Most studies found the presence of antibodies after the first dose, although in small amounts. Early appearance could be detected three days after vaccination up to six weeks in both first dose and second dose of vaccination. The highest levels and immune response were found about four weeks after vaccination, but at the second dose, the antibody appeared between days four and 10. After that, there may be a chance to experience a decline before the sixth week, especially on day 15 or day 43 ± 4. These studies stopped examining antibodies at the sixth week. Therefore, the data provided are limited to detection at the sixth week.

Comparison of antibody levels by type of vaccine

Not all studies address differences in antibody levels based on the type of vaccine received. There are four studies discussing antibodies in blood and breast milk samples. Research that discusses the comparison of antibodies in breast milk conducted by Lechosa-Muñiz et al. (2021),[23] Selma-Royo et al. (2021),[24] Gray et al. (2021)[25] and Valcarce et al. (2021).[52] The mean antibody titers found in breast milk were found to be different for each vaccine type, 0.41, 0.45, and 0.09 (AU) for mothers who received BNT162b2, mRNA-1273, and ChAdOx1-S (one dose), respectively. No differences could be found between those vaccinated with BNT162b2 vs. mRNA-1273.[23] Research conducted by Selma-Royo et al. (2021) found that mothers vaccinated with Moderna and BioNTech/Pfizer after the first dose had a higher increase in breast milk anti-SARS-CoV-2 IgG than Oxford/AstraZeneca ($p < 0.0001$) and BioNTech/Pfizer ($p = 0.002$). In addition, the number of doses received also determines the amount of antibodies present in the breastmilk. COVID-19 vaccination induced anti-SARS-CoV-2 IgA and IgG in breast milk with higher levels after the 2nd dose.[24] This is consistent with the study of Gray et al. (2021) who found that booster doses of the vaccine increased SARS CoV-2 IgG, but not IgA in breast milk.[25] Another study conducted by Valcarce et al. (2021) found that both mRNA vaccines produced statistically significant SARS-CoV-2-specific IgA and IgG in breast milk and plasma.[52]

In serum, when analyzing IgG, the mean antibody titres observed in the serum of lactating mothers were different according to the type of vaccine they received, being 0.32, 0.30, and 0.16 (AU) for mothers who received BNT162b2, mRNA-1273, and ChAdOx1-S (one dose), respectively. When analyzing IgA, the mean antibody titres observed in the serum of lactating mothers showed differences according to the type of vaccine administered: 0.12, 0.16, and 0.02 (AU) for mothers who received BNT162b2, mRNA-1273, and ChAdOx1-S (one dose) vaccines, respectively.[23] In the comparison of antibodies from samples in the maternal sera and umbilical cord in a study conducted by Gray et al. (2021) found that neutralizing antibody titers were lower in the umbilical cord than maternal sera, although this proved not to be statistically significant ($p = 0.05$). Vaccine booster doses increase SARS CoV-2 IgG, but not IgA, in blood.[25]

The mean of SARS-CoV-2 IgG in plasma was a significantly higher at post second vaccine dose in mothers vaccinated with Pfizer vs. mothers vaccinated with Moderna ($p = 0.005$).[52] The type of vaccine, the number of doses, and the location of sampling have a role in the number of antibodies found.

Discussion

The antibody status in the breast milk of breastfeeding mothers who have been exposed to COVID-19 or are highly suspected and those who have been vaccinated against COVID-19 differs. The dominant antibody in mothers infected with COVID-19 is IgA, while the predominant antibody in vaccinated mothers is IgG, according to Young *et al.* (2021).[18] Vaccination is associated with an increase in dominant IgG and IgA antibodies after direct viral infection. These results differentiate antibodies in breast milk between vaccinated and infected mothers with COVID-19. Breast milk from both groups, namely mothers who are infected with COVID-19 and mothers who are vaccinated showed neutralizing activity against live SARS-CoV-2 virus, which could be attributed to SARS-CoV-2 IgA and IgG antibodies.[18] The dominant IgA response in breast milk can be found according to previous studies, namely the result of natural infection.[26]

Antibody transfer through breast milk is an evolutionary strategy for enhancing immunity early in life.[27] Research conducted by Pullen *et al.* (2021) in which they applied a serologic system to characterize SARS-CoV-2 specific antibodies in maternal serum and breast milk found a preferential transfer of antibodies capable of causing neutrophil phagocytosis and neutralization. Distinct SARS-CoV-2-specific antibody response was observed in serum and breast milk from individuals previously infected with SARS-CoV-2, with a predominant transfer of IgA and IgM into breast milk.[27] IgA is the most essential class of Ig provided by breast milk to infants because it acts in the intestines while the function of secretory IgA (SIgA) in infants is still in development.[28] The presence of IgM antibodies was found in this study although not all studies examined the presence of IgM even if only in a small sample. The presence of IgM in some samples suggests the possibility that breast milk may have a protective effect on the newborn.[29]

Although IgG are present in breast milk, they are functionally attenuated.[27] Emerging data from vaccinated pregnant and lactating women suggest that vaccine-induced transfer may be altered due to unusually high levels of IgG antibody induced by mRNA vaccines approved by the current Emergency Use Authorization.[25] Vaccine gives the baby strong IgA and IgG antibodies and may increase immunity compared to natural infection.[27] Nevertheless, the IgG transfer scheme still needs to be studied further because this study has not been able to find out about the transfer mechanism. A study conducted by Golan *et al.* (2021) found that no IgG was found in the plasma of infants whose mothers were vaccinated during lactation.[30] Although high levels of IgG were found in breast milk, these antibodies may not be transferred effectively to the baby. IgA antibodies produced after vaccination with the Pfizer/BioNTech vaccine resist the gastric phase but are degraded during the intestinal phase of the infant's digestion. By contrast, IgG is more susceptible to degradation in both digestive phases.[31] The results of another study found that maternal SARS-CoV-2 IgG was efficiently transferred across the placenta when infection occurs more than two months before delivery. Passive immunity inherited from the mother can last in infants for up to six months. Neonates can mount a strong antibody response to perinatal SARS-CoV-2 infection.[32] Antibodies shown from breastfeeding may have a protective effect on the recipient infant, provided that the infant has not increased its immune response to infection.[33]

Studies showing the duration of antibody persistence in breast milk are limited. Antibodies can be detected in mothers who are breastfeeding and infected with COVID-19 as early as day 10 and can last for up to 10 months. IgA levels are known to decrease over time, while IgG is relatively stable. In lactating mothers who were vaccinated, the trial was only up to six weeks in duration. Antibodies have been detected after the first dose of vaccination for three days and can last up to six weeks. Before six weeks, it can decrease. It is not known whether the antibody status will still decrease or persist at a certain point. Individual research by Young *et al.* (2021) showed that IgG began to decline by 90 days after the second vaccine dose.[18] This suggests that further research is needed to investigate the exact duration of antibodies in breast milk after vaccination.

The type of vaccine is related to the number of antibodies detected in breast milk even though IgG status is more dominant than IgA and IgM. Post-vaccination IgG levels reached levels similar to those of terminally ill COVID-19 patients and demonstrated a decreased breadth of antibody responses targeting the endemic coronavirus.[34] Another study conducted by Dashdorj *et al.* (2021) found that IgG levels from high to low were Pfizer/BioNTech, AstraZeneca, Sputnik V and Sinopharm, respectively.[35] These different responses also allow different amounts of antibodies to be transferred into breast milk.

Based on the results of the opportunity for antibody transfer, breastfeeding is highly recommended for mothers infected with COVID-19 and mothers who have been vaccinated. Research conducted by Verd *et al.* (2021) found that in a sample of children visiting emergency services with potential symptoms of COVID-19, a higher prevalence of

positive SARS-CoV-2 reverse transcriptase (RT)-PCR test results among those who were formula-fed exclusively compared to those who have been breastfed. Breastfeeding may reduce the risk of exposure to COVID-19 and other infections in children, compared to formula feeding.[12] It is vital to continue to breastfeed according to the available time duration. Women with confirmed COVID-19 should be advised to adhere standard precautions for contact with breastfeeding.[36]

There are clear and up-to-date recommendations on the duration of breastfeeding from the American Academy of Pediatrics, UNICEF and WHO. WHO and UNICEF recommend: early initiation of breastfeeding within 1 hour of birth; exclusive breastfeeding for the first 6 months of life; and the introduction of complementary (solid) foods that are nutritionally adequate and safe at the age of 6 months along with continuous breastfeeding until the age of 2 years or older.[55,56] In the first 6 months this is important because the baby's production of sIgA is low during that period.[37] Apart from direct breastfeeding, every mother has the right to choose to breastfeed her baby and health workers need to help ensure good handwashing practices before and after expressing the milk.[38]

Limitations and recommendations

Most of these studies only involve a small number of samples, and they did not find a mechanism for antibody transfer during breastfeeding. We recommend that future research explores antibody testing with a more extended research duration (more than six weeks) in vaccinated postpartum women. In order to ensure that the reported time is more accurate, multiple individual studies that directly discuss the comparison of antibodies in vaccinated mothers with mothers exposed to COVID-19 and the assessment of antibody transfer mechanism by breastfeeding may be useful. In addition, the researcher was unable to compile a meta-analysis because it included various types of academic papers resulting in a lack of homogeneity.

The strength of this study is that there have not been many studies that have reviewed the comparison of antibody types and duration of antibodies in infected and vaccinated postpartum women. The results obtained can support evidence-based health promotion to support continued breastfeeding during the pandemic and allow for the right time to support antibody transfer.

Conclusions

IgA and IgG antibodies were found in the breast milk of infected and vaccinated postpartum women. Infection with SARS CoV-2 is related with the presence of IgA, but vaccination is associated with increased IgG. Antibody levels persisted from day 10 of onset to 10 months in infected breastfeeding mothers and start from three days to six weeks in vaccinated breastfeeding mothers. Antibodies produced in breast milk in infected and vaccinated postpartum women have different dominant types. Further research needs to be done regarding the mechanism of antibody transfer in breast milk, longer research duration and studies that directly examine the comparison of antibodies in breast milk in vaccinated and infected postpartum women.

Data availability

Underlying data

All data underlying the results are available as part of the article and no additional source data are required.

Reporting guidelines

Zenodo: PRISMA checklist for 'Records of antibodies in breast milk in postpartum women who have been vaccinated or exposed to COVID-19: A systematic review. https://doi.org/10.5281/zenodo.6785317.[39]

Acknowledgement

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Open Peer Review

Current Peer Review Status: ✔

Version 3

Reviewer Report 28 September 2022

https://doi.org/10.5256/f1000research.138773.r151059

✔ **Nicole Cacho** (iD)

Department of Pediatrics, Division of Neonatology, University of Florida, Gainesville, FL, USA

Authors Kurniawati and Rahmawati revised the manuscript as requested. Thank you for the opportunity to review this important work.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Breast milk and lactation research in the NICU

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 15 September 2022

https://doi.org/10.5256/f1000research.137772.r148869

? **Nicole Cacho** (iD)

Department of Pediatrics, Division of Neonatology, University of Florida, Gainesville, FL, USA

Thank you for the opportunity to review this revised version of the manuscript.

Authors Kurniawati and Rahmawati revised the original manuscript "Records of antibodies in

breast milk in postpartum women who have been vaccinated or exposed to COVID-19: A systematic review." In short, this study explored the potential immune protection provided in breast milk by examining 20 studies that focused on breast milk SARS-CoV-2 antibodies. The revised manuscript is now more comprehensive and will add to the literature on post infection and vaccination breast milk antibodies. There are a few points to be addressed prior to indexing.

1. Valcarce et al. did compare the levels of breast milk antibodies after vaccination of two separate vaccine types. The data can be found at the bottom of Supplementary Table 1.

2. In the manuscript when discussing antibodies, it's important to distinguish the type of antibody (serum vs. breast milk). For instance, in the section "Comparison of antibody levels by type of vaccine" there are three separate lines where the type of antibody is unclear. See lines 3, 8 and 11.

After these points are addressed, I would recommend the manuscript for indexing.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 17 Sep 2022

eighty mardiyan kurniawati, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

Dear Reviewer

Thank you for providing additional suggestions to improve this manuscript. We have revised the paragraph in the 'comparison of antibody levels by type of vaccine' section. Hopefully our revision is in accordance with the suggestions you give and can be approved. Thank you for your time.

Best regards
Eighty M.K

Competing Interests: No competing interest

Version 1

Reviewer Report 18 August 2022

https://doi.org/10.5256/f1000research.134202.r144205

Nicole Cacho [ID]

Department of Pediatrics, Division of Neonatology, University of Florida, Gainesville, FL, USA

Kurniawati and Rahmawati's interesting manuscript "Records of antibodies in breast milk in postpartum women who have been vaccinated or exposed to COVID-19: A systematic review" summarizes the various manuscripts on breast milk antibodies following COVID-19 exposures and vaccinations. It is obvious the authors worked hard to review the manuscripts and compose the manuscript.

The article highlighted the specific antibody findings of trends of which type of antibodies were more prevalent after vaccination compared to COVID-19 infection. This extensive work on reviewing 20 cohort studies has highlighted trends in antibodies and laid groundwork for future systematic reviews on studies with longer follow up and possible meta-analysis. Unfortunately, the differences in methods and lack of homogeneity made it impossible to perform a meta-analysis. This work is an important addition to the literature on breast milk immunity in the setting of a global pandemic with COVID-19.

Major Revisions include the following:
1. Overall, the grammar needs extensive editing.

   This is especially needed in the Introduction section. Many of the sentences below are confusing to the extent that I unsure of the meaning the author is trying to convey.

   Introduction section with confusing sentences (page numbers refer to the pdf version of the manuscript):
   Page 1, 1st paragraph, First line: ...and caused changes in the phases of life. In what ways?
   Page 1, 1st paragraph, 3rd sentence: Consider changing to New mothers and their infants are more susceptible to infection compared to the general population.
   Page 1, 2nd paragraph, "Breast milk is the only nutrient needed in the first 6 months of life." Did you mean only nutrition?
   Page 1, 2nd paragraph, "Breast milk can be an opportunity and hope for babies to achieve protection". Would take out hope to make it more scientific.
   Page 1, 2nd paragraph, "Breastfeeding can protect "you" for at least six months because breast milk contains..." Vague you is confusing. Instead would say "Breastfeeding can provide protection for at least the first 6 months because breast milk contains...
   Page 1, 3rd paragraph, The risk of being exposed to the virus and providing nutrition is a dilemma... What does this mean? Dilemma to whom? To the mom, or for the baby? Why?
   Page 1, 3rd paragraph, would not use "fake news" which is slang, instead keep the word misinformation.

   Discussion section sentences which need clarification
   Page 11, 1st discussion paragraph, "This varies greatly", What varies greatly?
   Page 11, 1st discussion paragraph, "Breast milk from both groups showed neutralizing

activity…" Which groups?

Page 11, 2nd discussion paragraph, "IgA is the most essential glass of Ig provided by breast milk…produced by infants is still in development." What is still in development?

"The presence of IgM in some samples (breast milk) suggests the possibility that breast milk may have a protective effect on the newborn." In what way in the context of this systematic review?

Page 11, 3rd paragraph, 3rd sentence, "It gives the baby strong IgA and IgG antibodies and may increase immunity comparted to natural infection." What is "it"? mRNA vaccines? If so, say these vaccines or mRNA vaccines.

Page 12 of discussion first sentence, "Needs to be studied further because different studies can find different results." This sentence is too vague. Do you mean that studies report conflicting results? If so, say that.

Page 12, 3rd paragraph , Dashdorj et al. 2021, which antibody?

2. Be consistent throughout the manuscript and tables when describing the population.

Determining the difference between exposed to COVID-19 or infected with COVID-19 (or even suspected infection with COVID-19). This point is important as many people that are exposed may not know they are exposed and if asymptomatic will likely not get tested. Thus, simply exposed does not seem like the description that describes the majority of the population in these 20 studies. Be consistent in the terms of infected or exposed. For example, the title of Table 2 is "Characteristics of research on mothers with COVID-19 and antibody status in breast milk" but on Page 4 under the section Data abstraction and synthesis, "Table 2 is antibody contact in mothers exposed to COVID-19" but later on Page 10 under Antibody status in breast milk from infected other." Table 2 disuses antibody status in mothers who had been infected with COVID-19. Based on the majority of the studies confirming COVID-19 with PCR (with the exception of 1 study by Bauerl et al. 2021 which had CPR and/or after COVID-19 infection), I would advise changing the title of Table 2 to "Characteristics of research on mothers infected with COVID-19 and antibody status in breast milk". Furthermore, I would not refer to Table 2 in the text as mothers who had been exposed.

3. Recommend removing or modifying certain points that are not necessary to the flow of the manuscript.

Page 1, 2nd paragraph, "Infants with fever have fever episodes of respiratory illness, which implies that breastfeeding may provide local mucosal protection?" I'm not sure what that means. Does this mean all infants with fever, or with COVID-19? If it's a general statement of all infants with fever of unknown cause, it does not add to the paragraph. If it's meaning infants with COVID-19 with fever, then say that.

Page 12, 4th paragraph, I would remove the sentence "Therefore, women with confirmed COVID-19 must adhere to standard precautions for contact with breastfeeding." Contact precautions were not previously discussed and may be confusing or send unclear messages. If it is strongly desired to keep this sentence, would change must to "should be

advised" and also cite WHO, CDC and AAP recommendations for precautions.

Page 12, 4th paragraph, I would update the sentence "The need to encourage breastfeeding may be justified at least during the first 6 moths of life, when the infant's secretory IgA production is insignificant." There are clear and updated recommendations on the duration of breastfeeding from American Academy of Pediatrics, UNICEF and WHO: which all say exclusive breastfeeding for first 6 months and then up to 2 years of age. You could then highlight the importance of the first 6 month based on infant's low production of sIgA after discussion the general breastfeeding duration recommendations.

WHO and UNICEF recommend:
- early initiation of breastfeeding within 1 hour of birth;
- exclusive breastfeeding for the first 6 months of life; and
- introduction of nutritionally-adequate and safe complementary (solid) foods at 6 months together with continued breastfeeding up to 2 years of age or beyond.
(9 June 2021)
https://www.who.int/news-room/fact-sheets/detail/infant-and-young-child-feeding

AAP supports continued breastfeeding, along with appropriate complementary foods introduced at about 6 months, as long as mutually desired by mother and child for 2 years or beyond. These recommendations are consistent with those of the World Health Organization (WHO). (See below for citation)

Joan Younger Meek, Lawrence Noble, Section on Breastfeeding; Policy Statement: Breastfeeding and the Use of Human Milk. *Pediatrics* July 2022; 150 (1): e2022057988. 10.1542/peds.2022-057988

4. Important to use language that qualifies interpretations of studies that suggest findings but not strongly. For instance, on Page 12, 4th paragraph, "Breastfeeding can reduce the risk of exposure to COVID-19 and other infections in children when compared to formula feeding (reference 12 by Verd et al. 2021)." This is too strong of a statement since the cohort was retrospectively reviewed in a secondary analysis and used "Any breastfeeding" vs. exclusive formula feeding and these were in children up to 13 years of age. The conclusion section uses the word suggest that there is protection with breastfeeding. Thus, I recommend changing the work "can" reduce to "may" reduce.

5. Important point to consider - Mechanism of antibody transfer in the breast milk. I am uncertain what the meaning of this statement is. As per World Health Organization, the presence of IgA in breast milk is one of the ways in which breastfeeding protects infants against infection and death. This appears to be a big part of the conclusion section for future direction but it is not clear that it is based on the results presented.

6. Tables

Table 1: The question of How precise are the results? Is not a yes/ no question. Per CASP checklist for cohort study the included HINT was Look for range of confidence interval. I would also include p values if given. If not available, list "no data available" for that particular study. This is important for the statistical analysis portion. After you can compare

and contrast studies within each group of vaccinated or infected mothers.

Table 2: To make it more consistent for no. of infected mothers, just include the numerical value, (two are in words).

Table 2: Bauerl et al. sampling time is listed as before and after. How many days or weeks? Also how is infection defined in this study, if not by PCR?

Table 2: last entry Demers-Mathieu et al lists sampling time as 16-84 days. What is that in relation to?

Table 2 Heading Duration time: What is this the duration of? Follow up with patients?

Table 3 Heading Time: of what? Timing after vaccination when antibody levels are measured?

Table 3: Perl et al: which antibodies under "Antibody status"?

It would be informative to see an addition of antibody breakdown by type added to Table 3.

7. Figures

Figure 1 is the Flow chart of the study selection but Figure 1 is mentioned under Results subheading Time duration of antibodies appearing and is described as showing "Duration of antibody time associated with PCR confirmation or symptoms, breast milk testing, and vaccine administration. Was this supposed to be labeled as Figure 2. If so, I didn't see a Figure 2.

Figure 1 Flowchart is confusing because the numbers do not add up as they are explained. It appears that from the 1465, 500 were eliminated to leave 965. Then another 935 were eliminated to leave 30. Next 10 were eliminated to ultimately leave 20 studies. Each elimination should be explained clearly and the numbers should match up. Minor point: From 30 to 20 in the last 2 boxes in the flowchart reports excluded explained 11 that were eliminated but 30-11=19, not 20. Please revise this flowchart using an example from another systematic review.

Small revision points:

In methods on keyword search, it seems that "breast milk" and "COVID-19" were typed twice as an error. Please fix this. These are details of the methods and analysis to allow replication by others.

What is meant by "unclear information was included in the exclusion criteria"? Is this for a few of the studies that were not included or is this describing the majority of the 20 studies that were included?

What is a funnel plot? If you mention it, you may want to explain it.

Page 10, last sentence, typo: COVaID-19 instead of COVID-19.

Page 11, Results section, Heading Antibody status in breast milk from vaccinated, second to last sentence, Serum levels in infants appears to only be included in one study, so please provide the reference for that study as a superscript and also mention it was only one of the 20 studies. Also, how many infants had serum levels? Not many mothers would want you to poke their babies to get a blood draw.

Page 11, Results section, heading time duration of antibodies appearing, Last sentence is an interpretation of results thus, belongs in discussion section (which you already included on page 12, 2nd paragraph), so you can delete this sentence.

Limitations and recommendations section: instead of the last sentence, "…because it included various types of academic papers." Consider saying lack of homogeneity.

Why do Pace et al. and Fox et al. not fit with other available evidence? This should be explained in the discussion section. Personally, from Table 2 the Demers-Mathieu et all paper has all 26 patients with all antibodies in milk including IgA, IgG and IgM. I don't believe those results. Do you?

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Are the rationale for, and objectives of, the Systematic Review clearly stated?
Yes

Are sufficient details of the methods and analysis provided to allow replication by others?
Partly

Is the statistical analysis and its interpretation appropriate?
No

Are the conclusions drawn adequately supported by the results presented in the review?
Partly

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 23 Aug 2022

eighty mardiyan kurniawati, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

Dear reviewers,

Thank you for being willing to review our manuscript and give suggestions to improve its quality. We've tried to revise it. I hope you are satisfied with our revision.

Thank you for your time.

Best regards

Competing Interests: There is no competing interest.

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