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Is there a reactive IgG antibody for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in maternal breast milk?

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Abstract

Restricted data on the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) reactive IgG antibodies by breast milk to infants, born to mothers who did not know if they had coronavirus disease 2019 (COVID-19), are presented. The objective of the present work was to demonstrate the existence of specific IgG antibodies against the SARS-CoV-2 virus in the milk of breastfeeding mothers in the suburban area of Şanlıurfa, Türkiye. This retrospective analysis examined the medical records of maternal and neonatal data for all 51 newborns to 50 mothers from September to October 2020. The present work was approved by the local medical ethics committee. Written informed consent was obtained from the mothers. Testing SARS-CoV-2 reactive IgG was performed using ELISA assay. SARS-CoV-2 reactive IgG were detected in 58% of breast milk. Over the past year, SARS-CoV-2 reactive IgG identified in breast milk of mothers without symptoms of viral respiratory infection (self-reported diagnosis) was 12.5 times lower than mothers with symptoms. The IgG responses were different between symptomatic and asymptomatic mothers' and infants' samples. These results propose that along with the COVID-19 pandemic, breastfeeding could be safe, and the existence of SARS-CoV-2-reactive IgG antibodies in breast milk could provide passive immunity to breastfed infants, and keep them safe against COVID-19 infection.

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Introduction

In December 2019, humans all around the world realised how their lives changed suddenly; their lives were in danger, and they were vulnerable. They soon realised that this situation was a pandemic, and they should fight against it by getting to know the virus and taking necessary precautions.

For the first time after unexplained pneumonia patient reports at the end of December 2019 in Wuhan City of China, the causative agent was determined to be coronavirus 2, and this disease was named COVID-19 by the World Health Organization (WHO). International Committee on Taxonomy of Viruses (ICTV) declared "severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)" as the name of the new virus in February 2020 (WHO, 2020a). The SARS-CoV-2 is a member of a large family of viruses with the inclusion of the common cold, SARS (severe acute respiratory syndrome), and MERS (Middle East respiratory syndrome) (Fan *et al.*, 2021).

While the pandemic caused by the SARS-CoV-2 continues to be a global public health problem, the question of whether the mother can protect the infant with her milk is important for breastfed babies, if the mother is infected with this virus.

In this context, considering that the number of cases including pregnant and breastfeeding women are increasing exponentially, breast milk for infant health is also a material that is open to research in order to prevent the spread of COVID-19. Laboratory tests to detect viruses or antibodies from the bodily fluids play important roles in confirming the diagnosis of COVID-19, identifying infected individuals, and defining immune metabolism (Lippi and Plebani, 2020; Marín Gabriel *et al.*, 2020). In the early stages of the pandemic, different strategies were considered on how to feed newborn babies by infected mothers. By keeping babies away from their mothers, the strategy of feeding babies with healthy milk instead of milk from their infected mothers was considered (Puopolo *et al.*, 2020). The present argument would remark that mothers should maintain

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to provide breast milk to their infants (Kimberlin and Puopolo, 2020). Science has continuously indicated that breast milk provides not only a range of nutrients for infant growth and development, but also various bioactive components including antibodies to protect infants against pathogens in the initial period of life (Yeung *et al.*, 2014).

The existence of IgM antibodies shows the early incidence of infection as IgG antibodies are an indicator of potential resistance or immunity. In the study on antibodies against the SARS-CoV-1 virus, it was stated that plasmas from SARS patients in the recovery period could be effective in the treatment of acute SARS patients, and that SARS-CoV-1 IgG and/or IgM antibodies might indicate passive immune antibodies that could provide a preventive or regenerative influence against the epidemic (Li *et al.*, 2003). Preventing or reducing the severity of the infant's disease by passing a protective antibody from mother to child through breastfeeding could also be achieved in this disease.

In one of the few studies conducted, it was reported that an infant born to a mother with COVID-19 had high IgM antibodies against SARS-CoV-2 (Dong *et al.*, 2020). Based on this report, the existence of SARS-CoV-2-reactive antibodies in breast milk could provide passive immunity to breastfed babies. However, the vertical migration of SARS-CoV-2, and the transplacental migration of antibodies remain a controversial issue (Zhu *et al.*, 2020; Zeng *et al.*, 2020a; 2020b); and nowadays, no effective oral antibody therapy has yet to be found for the infection. However, it has also been stated that breast milk antibodies which have highly multi- and cross-reactive features against SARS-CoV-2 and different coronaviruses, might be helpful to passivate and preserve against further coronavirus pandemics, exclusively in unprotected populations (Demers-Mathieu *et al.*, 2021). The present work demonstrated the existence of specific IgG antibodies against the SARS-CoV-2 virus in the milk of breastfeeding mothers, in the suburban area of Şanlıurfa, Türkiye.

Materials and methods

Ethical approval

Ethical approval was acquired from the clinical research ethics committee of Harran University, Şanliurfa, Türkiye (Protocol No.: HRU/20.17.04). The participants were informed about the objectives and secured privacy of the study, and while they accepted to participate in the study, they were also requested to sign a written consent agreement.

Demography of mothers

The first group of breast milk samples was gathered from 50 mothers throughout the COVID-19 pandemic (from September to October 2020). These mothers were asked about their COVID-19 condition, specific viral respiratory symptoms related to SARS-CoV-2 throughout last year, or if they have been exposed to people who were COVID-19 positive. No mother declared that she was sick while collecting the milk. The mothers brought their babies to the health centre for a routine vaccination schedule. The other group of breast milk samples was gathered from 12 mothers between January to June 2016. These breast milk samples were used as a control group.

Inclusion criteria were voluntary mothers between the ages of 18 to 45 who breastfed their infant, and lived in the suburban area.

Exclusion criteria were voluntary mothers below 18 or above 45, and live in the suburban area.

Collection of maternal information

A sociodemographic questionnaire was surveyed to the participants to supply information concerning their age, height, weight, number of children, date of birth of their infant, highest educational qualification, career, profession, and place of residence. Additional details were collected on the type of breastfeeding.

Presence of SARS-CoV-2 IgG antibodies in breast milk by ELISA

Breast milk samples (n = 50) were quickly dissolved at 37°C, and centrifuged at 3,000 rpm for 10 min at 5°C. After removing the upper layer of fat, the supernatant was used for ELISA. Test microplates (Nunc 269620, Denmark) were coated by 100 µL of whole SARS-CoV-2 virus in predetermined dilution made in coating buffer (pH 9.5), which was previously made by checkerboard, and incubated overnight at 4°C. Microplates were washed three times using PBS with 0.05% Tween-20 detergent. After washing with 100 µL of control and test milk samples, they were added to the wells, and incubated for 1 h at room temperature on a shaker. Then, after four times washing, the wells of the plates were added with 100 µL of conjugate (recombinant protein A/G peroxidase-conjugated; Pierce 32490), and plates were incubated on a shaker (44 rpm) for 1 h at room

temperature. After washing, all wells were added with 100 µL of a chromogenic substrate (10 mg OPD (*o*-phenylenediamine) tablet; P-8287, Sigma, St. Louis, MO, USA), in 25 mL of 0.05 M phosphate-citrate buffer with pH 5.5 and 10 µL of 30% H_2O_2. The reaction was stopped with 2 N H_2SO_4. The plates were shaken on an orbital shaker for 15 min prior to reading at OD490 nm using a microplate reader (VERSAmax 3.13/B2573).

Statistical analysis

Maternal and infant demographic and clinical properties were crosschecked with different factors. All analyses were conducted using SPSS 23.00 (SPSS Inc., Chicago, IL, USA). Phi correlation was used to explore the association between maternal and infant data. Two-sided $p < 0.05$ indicated significance. Average chart analyses were done using Stata 14 (StataCorp LP, College Station, TX). Data were transferred from Microsoft Excel (Microsoft Corp., Redmond, WA) into Stata 14 and reviewed for completeness.

Results

Sociodemographic information of mothers and infants

Analysis of the data based on the sociodemographic information of mothers and infants showed that the average age of the breastfeeding mothers was 27.78 ± 5.24, ranging between 19 - 42 years old. The average infant weight at birth was 2899 ± 543.92 g, ranging between 1900 - 4500 g. The infants in the present work were between the ages of 4 to 900 days, with average of 219.88 ± 229.54 days (Table 1). A total of 50 breastfeeding mothers were selected, comprising of nursing mothers who lived in the suburban area, and they filled out the questionnaires, Only 6% of breastfeeding mothers were active in business and well-educated, and the highest academic qualification achieved by breastfeeding mothers was bachelor's degree.

Table 1. Sociodemographic information.

	Positive	**Negative**
Age of mother (year)	27.82 ± 4.87	27.71 ± 5.96
Age of infant (day)	230.34 ± 233.51	205.42 ± 234.5
Number of pregnancy for mother	3.58 ± 2.21	2.71 ± 1.70
Birth weight of infant	2944.82 ± 571.08	2835.71 ± 525.15
COVID-19 test result	1	-
Mother showing symptom	1	2
Infant showing symptom	3	4
COVID-19 positive mother in the family	8	6
Mother who received influenza vaccination	2	-
Mother with chronic illness	3	2
Smoking mother	4	-

Breast milk testing

This retrospective analysis reported the medical data for maternal and infant, for all 51 infants born to 50 mothers, from September to October 2020. SARS-CoV-2 reactive IgG was detected in 58% of breast milk (Table 2). The antibody was not detected in any of the milk collected before the pandemic (Table 3). IgG in breast milk might have a cross-reaction with other coronaviruses and influenza viruses. Also, IgG, which is frequently produced at the termination of a recent infection, is exceptionally efficient at opsonisation and operating the supplement system, and frequently associated with immunity. SARS-CoV-2 reactive IgG in breast milk from influenza-vaccinated mothers were positive in the present work. Hence, more research is required to investigate the cross-reactive capability of breast milk antibodies to deactivate SARS-CoV-2 and others. Identically, SARS-CoV-2 reactive IgG in breast milk was lower in mothers (2%) without symptoms of viral respiratory infections in the last ten months, as compared to mothers with infections (56%). SARS-CoV-2 reactive IgG detected in breast milk from mothers that had symptoms (self-reporting diagnosis) throughout last year was 12.5-fold higher than mothers without symptoms in the past ten months. In

Table 2. Characteristics of IgG detected from mothers and infants.

Age of mother (year)	Age of infant (day)	Number of pregnancy for mother	Birth weight of infant (g)	COVID-19 test result	Mother showing symptom	Infant showing symptom	COVID-19 positive mothers in the family	Mother who received influenza vaccination	Mother with chronic illness	Smoking mother
24	180	2	2900	No	No	Fever	Yes	No	No	No
30	510	5	2000	No	No	No	No	No	No	No
35	150	7	3500	No	No	No	Yes	No	Hypertension	Yes
25	540	2	4500	No	No	No	No	No	No	Yes
22	60	1	3300	No	Cold	No	No	No	No	No
22	270	2	3700	No	No	No	No	No	No	No
26	540	2	2100	No	No	No	No	No	No	No
19	60	2	2400	No	No	No	No	No	No	No
27	30	3	2800	No	No	No	No	No	No	No
40	900	7	2500	No	No	No	No	No	No	No
22	180	3	2600	No	No	No	No	No	No	No
30	90	9	2000	No	No	No	No	No	Epilepsy	No
25	90	4	3000	No	No	No	No	No	No	No
32	7	5	2500	No	No	No	No	No	No	No
24	9	2	3400	No	No	Fever, Cold	No	No	No	No
24	180	1	3000	No	No	No	No	Yes	No	No
27	30	6	3300	No	No	No	No	Yes	No	No
34	30	4	3300	No	No	No	Yes	No	No	No
27	210	7	3200	No	No	No	No	No	No	No
25	120	1	3200	No	No	Fever	Yes	No	No	No
25	4	4	3400	No	No	No	No	No	No	Yes
37	120	2	3000	No	No	No	No	No	Asthma	Yes
30	300	4	3600	No	No	No	No	No	No	No
30	270	7	2600	No	No	No	Yes	No	No	No
30	720	4	2000	No	No	No	No	No	No	No
30	120	3	2800	No	No	No	Yes	No	No	No
27	540	2	3200	No	No	No	Yes	No	No	No
33	60	1	2800	No	No	No	No	No	No	No
25	360	2	2800	No	No	No	Yes	No	No	No

Table 3. Characteristics of IgG not detected from mothers and infants.

Age of mother (year)	Age of infant (day)	Number of pregnancy for mother	Birth weight of infant (g)	COVID-19 test result	Mother showing symptom	Infant showing symptom	COVID-19 positive mothers in the family	Mother who received influenza vaccination	Mother with chronic illness	Smoking mother
27	510	4	1900	No	No	No	No	No	No	No
23	240	1	2500	No	No	Fever	No	No	No	No
21	900	2	2000	No	Headache, fever	No	No	No	No	No
26	60	3	3400	No	No	Cough	No	No	No	No
25	120	2	3000	No	No	No	No	No	No	No
28	17	2	3000	No	No	No	No	No	No	No
30	7	4	3100	No	No	No	No	No	No	No
36	23	5	3200	No	No	Cough	No	No	No	No
18	270	1	2200	No	No	No	Yes	No	No	No
23	120	2	3000	No	No	No	No	No	No	No
21	180	1	2100	No	No	No	No	No	No	No
32	30	3	4000	No	No	No	No	No	No	No
38	90	7	2500	No	No	No	No	No	No	No
31	450	1	3500	No	No	No	Yes	No	No	No
42	180	6	2700	No	No	No	Yes	No	Asthma	No
21	7	1	2800	No	No	No	No	No	Hepatitis	No
26	630	2	3150	No	No	No	No	No	No	No
30	120	2	3300	No	No	No	No	No	No	No
27	60	2	2700	Yes	Fever, cough, muscle pain	Fever	Yes	No	No	No
30	240	4	2700	No	No	No	Yes	No	No	No
27	60	2	2800	No	No	No	Yes	No	No	No

one of these mothers, the COVID-19 antigen by RT-PCR from a nasopharyngeal sample, which was performed by the Ministry of Health, came out positive. Only one of these symptomatic mothers had positive IgG antibodies in her milk. Symptoms of viral respiratory infections were observed in seven (14%) of the infants, and antibodies were detected in three (6%) of the breast milk given to these infants. Viral respiratory infection symptoms were observed in three (6%) infants of mothers with IgG antibodies in their milk, and symptoms were observed in four infants from mothers without IgG antibodies in their milk. COVID-19 was diagnosed in the families of eight (44%) of the mothers, and we detected antibodies in their milk. The number of mothers in the risk group with chronic disease was five (10%), and we detected antibodies in the milk of three (6%) of these mothers who have not had symptoms of a viral respiratory infection (self-reporting diagnosis), and none of these mothers were influenza vaccinated. Four mothers were smokers, and we detected SARS-CoV-2 reactive IgG in all their milk, and one mother with chronic illness had family members who were diagnosed with COVID-19.

Discussion

Infants rely on breast milk from their mother as their only nutritional source (Emmett and Rogers, 1997). Human breast milk is the most bio-available food source for babies, supplying the hormones and immunological factors that shield infants from possible disease-causing agents (Verduci *et al.,* 2020). Although ten months have passed since the start of the pandemic, there is still restricted data on the potential transmission of the infection from mother to child, especially through breastfeeding. In May 2020, WHO reported new guideline on clinical management of COVID-19, proposing private breastfeeding for at least in the early stages of lactation and breastfeeding, beside supplementary aliments up to two years of age as handling essential precautions for infection protection in infants born to mothers who were suspected or confirmed with COVID-19 (WHO, 2020b). Breastfeeding protects infant from infections primarily *via* secretory antibodies. In the early stages of lactation, IgG has anti-inflammatory factors, and provides passive immunity, which protects them from reinfection (Nimmerjahn, 2014). In the present work, we determined the antibody (IgG) to SARS-CoV-2 in breast milk by home-made ELISA. Fifty breast milk samples from different mothers were only assessed for the existence of IgG antibodies, with 29 breast milk samples having specific IgG antibodies for SARS-CoV-2. Some mothers might have been exposed to SARS-CoV-2, and some of them might not want to declare themselves as infected with SARS-CoV-2 for various reasons, or they can be asymptomatic carriers. The latter is important because such infections are widespread (Guan *et al.,* 2020). In a recent study, SARS-CoV-2-specific IgG (on days 8 and 24) was reported in only one breast milk from a mother who was positive for SARS-CoV-2 (Preßler *et al.,* 2020). In another study, a total of 41 women supplied breast milk samples for ELISA, and 58.5% of the sample having specific IgG antibodies for SARS-CoV-2 (Demers-Mathieu *et al.,* 2021).

Conclusion

The IgG responses were different between symptomatic and asymptomatic mothers' samples. The presence of SARS-CoV-2-reactive antibodies in breast milk could provide passive vaccination to babies. There is currently no effective oral antibody treatment for COVID-19 infections. Breast milk antibodies are cross-reactive against SARS-CoV-2. It can be useful for deactivating the CoV antigen, especially in unprotected people. Intensive studies are needed for antibodies reactive to SARS-CoV-2 to be purified from breast milk and used in the medical field.

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