Original article

SARS-CoV-2 spike RBD-specific IgA and IgG antibodies in breast milk after vaccination with the protein subunit vaccine Abdala

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ABSTRACT

Background: COVID-19 vaccines that trigger a strong secretory antibody response in breast milk may achieve effective passive protection of vulnerable newborns and breastfed infants of immunized mothers. The aim of this work was to investigate the presence of SARS-CoV-2 spike RBD-specific IgA and IgG antibodies in breast milk, 5 and 9 weeks after vaccination with 3 doses of the protein subunit vaccine Abdala, compared to those found in breast milk from COVID-19-recovered women, collected at least 40 days after the infection.

Methods: SARS-CoV-2 spike RBD-specific IgA and IgG antibodies were semi-quantified by indirect ELISA, using a homemade standard generated by pooling twenty breast milk samples with high absorbance values according to preliminary data. The validity of the standard curves was proved following the European Medicines Agency Guideline. Two breast milk samples from 2 unvaccinated women who had not been infected with COVID-19 were included as negative controls. Potentially neutralizing antibodies were assessed by a SARS-CoV-2 surrogate virus neutralization test.

Results: High levels of anti-RBD IgA antibodies were detected in breast milk samples 9 weeks after vaccination and anti-RBD IgG antibodies rise from the fifth to the ninth week. In the post-COVID-19 time that was evaluated, the IgG-type response was notably higher compared to both post-vaccination periods. Neutralizing antibody titers were similar in breast milk from vaccinated and COVID-19 recovered women.

Conclusions: This is the first report about the immune response in breast milk after the administration of a COVID-19 protein subunit vaccine, which could provide analogous protection to that conferred by SARS-CoV-2 infection. This implies a potential passive immunity that breastfed infants receive from their mothers vaccinated with Abdala.

1. Introduction

In the combat against the COVID-19 pandemic, scientists around the world have achieved, like never before, the rapid development of new vaccines. Vaccines are the most valuable tool against SARS-CoV-2; since they have slowed the spread of this virus and give hope to eliminate it soon. Several technology platforms have been applied to develop safe and effective vaccines. The most used have been the nucleic acid vaccines, (DNA and RNA-based vaccines); mRNA vaccine production has fewer biosafety issues, making them quicker to produce. Other platforms, such as conventional whole virus vaccines (inactivated or live attenuated), viral vector vaccines and recombinant protein-based vaccines (protein subunit vaccines, virus-like particles) have also been tested [1,2].

Cuba initiated the vaccination program against COVID-19 on July 29, 2021. The Center for State Control of Drugs, Equipment and Medical Devices (CECMED) granted the authorization for emergency use to the Cuban
vaccine Abdala on July 9. Abdala is a protein subunit vaccine based on the recombinant RBD of SARSCoV-2 spike protein produced in *Pichia pastoris*. The sequence of the RBD antigen was equal to that of the SARS-CoV-2 Wuhan-Hu-1 strain (NCBI Acc. No.YP_009724390) [3].

The complete vaccination schedule of Abdala includes 3 doses, administered every 14 days. In Cuba, health care workers were the first groups who received the vaccine. The vaccine was safe, well tolerated and induced humoral immune response against SARS-CoV-2 [3]. Since no pregnant and lactating women were included in the safety and efficacy trials, the CECMED authorized the vaccination of this population in a second stage, after the demonstration of safety, tolerability and immunogenicity of the Abdala vaccine.

COVID-19 vaccines that trigger a strong secretory antibody response in breast milk may achieve effective passive protection of vulnerable newborns and breastfed infants of immunized mothers, if they are not old enough to be vaccinated [4]. Breastfeeding has been correlated with a lower risk of mucosal defense-related infections, mainly respiratory infections. This mucosal defense is mainly due to passively transferred breast milk-derived IgA providing partial mucosal immune protection in breastfed infants [5,6].

Observational studies have shown that vaccination in pregnant and lactating women generated a robust humoral immunity and the immune transfer of neutralizing antibodies from mother to neonates and infants. Both transplacentally-derived maternal IgG specific to SARS-CoV-2, as well as breast milk-derived IgA and IgG, protect them against COVID-19 infection. Most of these studies were done on mRNA vaccines [7]. But, to date, there are no published data related to the immune response in breast milk after vaccination with protein-based vaccines.

In this work we investigated the presence of SARS-CoV-2 spike RBD-specific IgA and IgG antibodies in breast milk, 5 and 9 weeks after the vaccination with 3 doses of the protein subunit vaccine Abdala. We compared breast milk antibody levels from vaccinated women to those found in naturally immunized women after SARS-CoV-2 infection. Potentially neutralizing capacity of anti-RBD antibodies was also assessed in breast milk samples.

2. Materials and methods

2.1. Study participants

This study evaluated anti-SARS-CoV-2 antibodies in independent breast milk samples collected from lactating women between September and November 2021. All samples were collected at the Neonatology Service of the General Hospital “Camilo Cienfuegos”, Sancti Spiritus, Cuba. Breast milk samples were obtained from 103 lactating women, who had never been infected with COVID-19 and who were vaccinated during pregnancy: 50 samples were collected 5 weeks after the third dose of the Abdala vaccine and 53 were obtained 9 weeks after. Fifty-two COVID-19-recovered non-vaccinated participants were also included, whose breast milk samples were collected at least 40 days after the positive RT-PCR test. Two breast milk samples from unvaccinated women who were never infected with COVID-19 were used as negative controls. Characteristics of the study participants were obtained by a questionnaire.

2.2. Breast milk processing

Breast milk samples were frozen and transported in a box with dry ice to the Center for Genetic Engineering and Biotechnology (CIGB) of Sancti Spiritus. Frozen samples were rapidly thawed at 37 °C and centrifuged at 1300 g for 15 minutes at 4 °C. The aqueous phase was separated from the fat layer using a micropipette with yellow tip. This aqueous phase was aliquoted into cryogenic vials and stored at -20 °C until analysis.

2.3. Detection of SARS-CoV-2 spike RBD-specific IgA and IgG antibodies by ELISA

Costar 3590 high binding polystyrene 96-well microtiter plates were coated with 100 μL/well of 10 μg/mL of recombinant SARS-CoV-2 RBD (CIGB, Havana, Cuba) in 0.1 M of sodium carbonate-bicarbonate pH 9.8, 2 hour at 37 °C. Coated plates were blocked with dilution buffer (DB), consisted of 1% (w/v) bovine serum albumin in phosphate buffered saline (PBS), for 1 hour at 37 °C, and washed once with 0.1% (v/v) of Tween-20 in PBS. Breast milk samples and negative controls were appropriate diluted with DB and incubated on the plate for 1 hour at 37 °C. Plates were washed 3 times and bound IgG and IgA were detected with horseradish peroxidase conjugated anti-human IgG (Dako) and anti-human IgA CBSSlgAH-HRP (CIGB, Sancti Spiritus, Cuba), respectively. After 4 washes, bound antibody was revealed using 0.6 mg/mL ortho-phenylenediamine in citrate buffer (pH 5.0) with 0.015% hydrogen peroxide, for 30 minutes in the dark at (22–25) °C. The reaction was stopped with 2.66 M sulfuric acid. The absorbance (A492nm) was measured by a plate reader (Labsystems Multiskan Plus) at 492 nm.

2.4. Semi-quantification of SARS-CoV-2 spike RBD-specific IgA and IgG antibodies by assignment of arbitrary units

Since no commercial standard was available in our laboratory, a homemade standard was prepared for the assignment of arbitrary units (AU), as a semi-quantification method of anti-RBD IgA and IgG antibodies in breast milk samples. This standard consisted of the mixture of ten breast milk samples from vaccinated mothers and ten
from mothers recovered from COVID-19. For this semi-quantification of SARS-CoV-2 spike RBD-specific IgA and IgG antibodies, it was applied the ELISA described previously. As assay blank was used the DB, since its background signal was the same of negative samples. An AU of 1000 was assigned to the highest A492nm value corresponding to the undiluted standard. The standard curves Log AU (IgA or IgG) versus A492nm were performed with ten 2-fold serial dilutions of standard with DB, spanning a range from 1000 to 1.95 AU. These sequential dilutions were added in triplicate (100 µL/well). The curves were fitted by a nonlinear, asymmetric sigmoidal 5-parameter logistic regression. The resulted fit was used to interpolate the AU of anti-SARS-CoV-2 IgA and IgG antibodies of each sample. To evaluate the validity of the standard curves, 6 ELISAs were performed: in 3 of them, the antihuman IgG conjugated with horseradish peroxidase was used, and in the rest, the antihuman IgA CSSigAH-HRP was used. The intra-curve accuracy was assessed for percent of relative error (%RE), which should be less than 20% in each dilution point [8]. The precision and accuracy inter-curves were evaluated by the coefficient of variation expressed as percentage (%CV) and %RE, respectively, which should be less than 20% in each dilution point [8].

2.5. Parallelism test between the homemade standard and the breast milk samples

This test was performed through the ELISA described previously, but the preparation of the samples was different. Six pools of 5 breast milk samples each were prepared: 3 pools from vaccinated mothers and 3 from COVID-19-recovered mothers. The pools and the homemade standard were diluted serially 1:2 with DB. Linear regression of data from back-calculated AU was fitted also by a 5-parameter logistic regression. Data precision (%CV) of each dilution point should not exceed 30% [8].

2.6. SARS-CoV-2 surrogate virus neutralization test

In this test, 20 samples with the highest IgA and IgG AU values per group were used. The neutralizing potential of breast milk antibodies against the SARS-CoV-2 RBD was determined by a SARS-CoV-2 surrogate virus neutralization test (sSVNT) [9] with some modifications. Costar 3590 high binding polystyrene 96-well microtiter plates were coated with 100 µL/well of hFc-ACE-2 recombinant protein expressed in HEK293T (Center for Molecular Immunology, Havana, Cuba), at 5 µg/mL in PBS, pH 7.4, for 3 hour at 37 °C. After a wash with 0.1% (v/v) of Tween-20 in PBS, the plates were blocked with DB for 1 hour at 37 °C. The breast milk samples, in 8 serial 4-fold dilutions with DB, as well as the negative controls, were pre-incubated (v/v) with RBD-HRP conjugated, diluted 1:32,000 with DB, 1 hour at 37 °C. The RBD-HRP conjugate, pre-incubated only with DB, was used as the positive control of the RBD-ACE-2 reaction. After the pre-incubation period, 100 µL/well of the mixtures of breast milk-RBD-HRP conjugate and 100 µL/well of the positive control were added to hFc-ACE-2 blocked plate and incubated 1 hour at 37 °C. The plate was washed 4 times and kept for 20 minutes in the dark at (22–25) °C with 100 µL/well of 0.6 mg/mL ortho-phenylenediamine, dissolved in phosphate-citrate buffer (0.2 M phosphate, 0.1 M citrate, pH 5.0) and 0.006% (v/v) hydrogen peroxide. The reaction was stopped with 100 µL of 2.66 M sulfuric acid and the absorbance was measured at 492 nm.

The sVNT cut point was defined as the A492nm value below which the samples were considered inhibitory of the RBD-ACE-2 reaction. The neutralizing antibody titer of each sample was determined by interpolation of the cut point in the sample dilution curve, fitted by a 5-parameter logistic regression. The cut point (CP) was calculated by the formula:

\[ CP = m(A492nm) - t(0.99; \text{one tailed}) \times SD \]

Where: m (A492nm) was the mean of 8 A492nm values from the positive control; SD was the standard deviation.

2.7. Statistical analysis

Curve fitting and statistical analyses were performed using the GraphPad Prism software, version 8.0.2 for Windows. Before evaluating the data for comparison, their normality was determined using the Shapiro-Wilk test (p < 0.05). The Kruskal-Wallis test was used to compare IgA and IgG AU and neutralizing antibody titers between the 3 groups of independent breast milk samples. As a complement to this test, if the null hypothesis was rejected, the Mann-Whitney test (2-tailed) was performed between 2 groups, with p < 0.05 to assess statistical significance.

3. Results

Breast milk was collected from 155 participants, 66.45% of them were vaccinated lactating mothers who had never been infected with COVID-19 and the rest were unvaccinated and recovered from this disease. The age of the participants, the gestational age and the childbirth were similar in both groups, as shows the Table 1. Regarding the health condition of the participants, differences were observed between the groups. Most of the vaccinated were healthy women; however, 67.31% of mothers from the COVID-19-recovered group had at least a chronic disease and required hospitalization after the RT-PCR-positive test, but only 4 of them had a severe infection. Asthma and arterial hypertension were the most prevalent diseases in both groups of participants. Clinical details of the COVID-19-recovered and vaccinated mothers are available in Table 1.
In preliminary ELISAs, the A492nm signals corresponding to SARS-CoV-2 spike RBD-specific IgA and IgG antibodies were detected in all breast milk samples from lactating women who received the Abdala vaccine or who were infected with SARS-CoV-2. The A492nm values from the negative samples were like the assay blank (DB). The homemade standard was prepared by mixing a total of twenty human milk samples with highest A492nm signal: 10 from vaccinated mothers and 10 from mothers recovered from COVID-19. The Fig. 1 presents the standard curves log AU (IgA or IgG) versus A492nm, achieved with ten 2-fold serial dilutions of the standard, which spanned a range of IgA and IgG AU from 1000 to 1.95. The curves fulfilled the acceptance criteria for the intracurve accuracy and for the precision and accuracy inter-curves (Table 2).

A linear regression was applied for the evaluation of the parallelism between the standard and the sample pools used for this test. The Fig. 2 shows that sample pool dilutions were parallels to each other and to the standard. Data precision of the dilution series into the AU range did not exceed 21.29% and 22.95% for IgA and IgG isotypes, respectively, and they fulfilled the acceptance criterion of the assay.

The inclusion of the standard curves in each ELISA allowed the interpolation of AU of SARS-CoV-2 spike RBD-specific IgA and IgG antibodies of all the breast milk samples. This semi-quantification method demonstrated that the AU of IgA and IgG antibodies differed between vacci-

IQR, interquartile range; SD, standard deviation.

| Characteristics                      | Vaccinated mothers (N = 103) | COVID-19-recovered mothers (N = 52) |
|--------------------------------------|-------------------------------|-------------------------------------|
| Age, median (IQR), years             | 26 (21-32)                   | 25 (21-29)                          |
| Mother’s health condition            |                               |                                     |
| Healthy, %                           | 78.64                        | 32.69                               |
| With chronic diseases, %             | 21.36                        | 67.31                               |
| Most prevalent chronic diseases, n   |                               |                                     |
| Arterial hypertension                | 8                            | 8                                   |
| Asthma                               | 5                            | 12                                  |
| Obesity                              | 3                            | 4                                   |
| Epilepsy                             | 2                            | 1                                   |
| Hypothyroidism                       | 2                            | 3                                   |
| Diabetes mellitus type 1             | 0                            | 1                                   |
| Cardiovascular disease               | 1                            | 2                                   |
| Others                               | 1                            | 4                                   |
| Parity primiparous, %               | 44.66                        | 34.62                               |
| Childbirth                           |                               |                                     |
| Vaginal delivery, %                  | 41.75                        | 42.30                               |
| Instrumental delivery, %             | 32.04                        | 34.62                               |
| Caesarean delivery, %                | 26.24                        | 23.08                               |
| Gestational age, mean ± SD, weeks    | 38.65 ± 1.33                 | 38.45 ± 2.32                        |

Table 1
Characteristics of the study participants.

Table 2
Evaluation of the acceptance criteria for the standard curves.

| Acceptance criteria     | IgA standard curve | IgG standard curve |
|-------------------------|--------------------|--------------------|
| Intra-curve accuracy    | 2.61 < RE(%) < 13.95| 0.11 < RE(%) < 12.43|
| Inter-curve precision   | 1.48 < CV(%) < 10.17| 5.70 < CV(%) < 13.68|
| Inter-curve accuracy    | 2.60 < RE(%) < 8.21 | 1.92 < RE(%) < 12.03|

CV (%), coefficient of variation expressed as percentage; RE (%), relative error expressed as percentage.
nated and COVID-19-recovered mothers (Fig. 3). A significantly higher reactivity of IgA antibodies was observed in most of the samples obtained 5 weeks after vaccination compared to the COVID-19-recovered participants (p < 0.0001), whose samples were collected at least 40 days after COVID-19 infection, that is, approximately, the same first post-vaccination time that was being evaluated. The IgA AU found in breast milk samples collected at 9 weeks after vaccination declined respect to the fifth week but were still significantly higher (p < 0.0001) than IgA AU of breast milk samples obtained from COVID-19-recovered mothers. It should be noted that 9 weeks after vaccination with Abdala the mean of IgA AU was around 500, which is the middle of the range of AU established for the assay.

In the group of vaccinated mothers, the IgG AU increased markedly (p < 0.0001) in the ninth week respect to the fifth one (Fig. 3). In the post-COVID-19 time that was being evaluated, the IgG-type response was notably higher compared to both post-vaccination periods (p < 0.0001).

Both vaccination and COVID-19 infection resulted in breast milk with neutralization activity detectable by sVNT (Fig. 4). The 80% and 85% of the evaluated samples, collected 5 and 9 weeks after vaccination respectively, were considered neutralizing of the RBD-ACE-2 reaction, since their A492nm values were below the cut point determined for each assay. These neutralizing antibodies were also detected in 80% of evaluated samples from COVID-19-recovered mothers. None of the negative control samples was sVNT positive.

To further interpret the sVNT data, we assessed neutralizing antibody titers per sample, which did not differ between the vaccinated and COVID-19-recovered groups (p = 0.122). Sixteen samples had titers very close to or equal to 65536, the upper dilution factor (Fig. 4): these higher neutralizing antibody titers were found in 4 (20%) breast milk samples obtained 5 weeks after vaccination and in the same number from COVID-19-recovered participants. The number of samples with titers close to or equal to 65536 doubled 9 weeks after vaccination (Fig. 4). All of these samples with higher neutralizing antibody titers were obtained from lactating mothers aged less than 24 years.

4. Discussion

While the anti-SARS-CoV-2 vaccinated population is increasing progressively around the world, a problem remains in the fight against COVID-19: newborns and infants are unvaccinated yet. However, as anti-SARS-CoV-2 antibodies can be passed to infants through breast milk, they might achieve protection against the virus if they are fed with breast milk from vaccinated mothers or from mothers who were COVID-19-infected [7,10].

COVID-19 vaccination is more recommended if carried out during pregnancy, because in this period occurs the passive transfer of antibodies to the fetus bloodstream. Immune transfer of COVID-19 neutralizing antibodies from mother to neonates has been detected via placenta and breast milk [11]. Mothers vaccinated during lactation only transfer antibodies to their infants through breast milk, which is an important component of the mucosal immunity [6]. Antibody-mediated protection provided by breast milk in breastfed infants is not expected to extend much beyond breastfeeding, because mucosal IgA antibodies do not have the durability of IgG antibodies in the blood [12]. Even so, the protection that this vulnerable group receives from breast milk containing anti-SARS-CoV-2 antibodies is not negligible.

This work demonstrated the presence of SARS-CoV-2 spike RBD-specific IgA and IgG antibodies in breast milk from lactating women who were infected with SARS-CoV-2, or who received the complete vaccination schedule of the Abdala vaccine during pregnancy. Due to the lack of a commercial standard for the quantification of both antibody isotypes, it was necessary to prepare a mixture of breast milk collected from vaccinated and from COVID-19-recovered lactating mothers, as standard for semi-quantification by assignment the AU of IgA and IgG antibodies per sample. This method allowed comparison of antibody levels between samples.
Fig. 3. SARS-CoV-2 spike RBD-specific IgA and IgG antibody response in independent breast milk samples from 3 groups of participants: mothers vaccinated with 3 doses of the recombinant protein-based vaccine Abdala (5 and 9 weeks after vaccination) and from COVID-19-recovered mothers. The Mann-Whitney test (2-tailed) was performed between 2 groups, in the 3 possible combinations, with \( p < 0.05 \) to assess statistical significance of the differences in the Arbitrary Units of IgA and IgG antibody isotypes. Data is presented as mean and 95% of CI of the antibody Arbitrary Units. Asterisks represent \( p\)-value <0.0001.

Fig. 4. Neutralizing anti-RBD antibody titers detected by a SARS-CoV-2 surrogate virus neutralization test. The assays were performed with a total of 60 breast milk samples collected 5 and 9 weeks after vaccination and from COVID-19-recovered mothers. Twenty samples of each type were tested. Data are presented as mean and 95% of CI of the neutralizing antibody titers.

The parallelism between this homemade standard and the samples was assessed. The goal of parallelism is to demonstrate that the sample dilution curve is parallel to the standard dilution, thus confirming that the standard is suitable for measurement of the endogenous analyte. Some factors may account for non-parallelism between standard and serially diluted samples. For instance, the standard may have an antibody population with binding features different from those of a serially diluted sample [13]. The parallelism was evaluated using 3 pools of each type of samples. The use of sample pools avoided the generation of multiple values for individual samples [14]. Parallelism was verified within samples as well as between samples and the standard after several dilutions. Although the standard curves fulfilled the acceptance criteria for precision and accuracy, the demonstration of parallelism confirmed the validity of these curves for the assignment of AU to all types of breast milk samples included in this study.

The highest percentage of participants with chronic diseases was found in the group of mothers recovered from COVID-19. Smith [15] stated that the immune response to vaccination in patients with chronic conditions is weaker. Soegiarto et al. [16] proved the correlation of hypertension with low antibody response following vaccination with inactivated SARS-CoV-2. Our results showed that the levels of AU of RBD-specific IgA antibodies was significantly lower in breast milk from COVID-19-recovered mothers, which may be a sign of the neg-
ative effect of chronic diseases in the level of this immunoglobulin, taking into account the findings of Fox et al. [17] who explained that spike-specific IgA titers persisted for as long as 10 months in breast milk of lactating women with a confirmed SARS-CoV-2 infection but without chronic health conditions affecting their immune system. However, in our study, the levels of RBD-specific IgG antibodies were not conditioned by the presence of a chronic disease, since these levels were significantly higher in COVID-19-recovered mothers compared to the vaccinated groups, which had the highest percentage of healthy participants.

Most of breast milk samples collected 5 weeks after vaccination with Abdala having around 800 AU of anti-RBD IgA antibodies, but a follow-up analysis revealed that the IgA levels declined after 9 weeks, although the mean of IgA AU were still around the half of the AU range stated for the assay, and we considered that it could be a valuable mucosal immune response at that post-vaccination time. Secretory IgA is the main antibody isotype in breast milk. This immunoglobulin is essential in protecting the mucosal surfaces of the infants by intracellular neutralization, immune exclusion and virus excretion [18].

Kelly et al. [19] also identified a gradual decline in anti-spike IgA in breast milk over time after the second dose of Pfizer-BioNTech/BNT162b2 vaccine. The reduction of IgA levels could be correlated to its natural kinetics [20]. While the kinetics of IgA and IgG against SARS-CoV-2 has been well studied in breast milk after the disease, there is a limitation of not fully understanding their dynamic after vaccination [21]. Our findings stand in line likewise with Valcarce and colleagues [22], who detailed the high IgA immune response in breast milk from mothers vaccinated with Moderna and Pfizer/BioNTech, which unlike Abdala, are mRNA-based vaccines. Juncker et al. [23] informed that, over a period of 70 days, the level of SARS-CoV-2-specific IgA in breast milk was comparable after vaccination and infection.

A significant anti-SARS-CoV-2 spike RBD-specific IgG levels in breast milk after the administration of mRNA vaccines was reported by Young et al. [24] and they referred that infection was related with a robust IgA response. Gray et al. [11] and Baird et al. [25] also revealed higher levels of anti-SARS-CoV-2 spike RBD-specific IgG in breast milk after vaccination. Jayathilaka and colleagues [26] affirmed that vaccines had significantly less IgA type-response to SARS-CoV-2, but comparable IgG responses those with natural infection, which does not agree with our results.

The study of SARS-CoV-2 spike RBD-specific IgG antibody levels in breast milk after administering a COVID-19 protein subunit vaccine has not yet been described. In this work, the IgG AU augmented from fifth to ninth week after vaccination with Abdala protein subunit vaccine. But those levels could rise in subsequent weeks; hence, further studies are needed to follow the increase in IgG AU over time in breast milk from mothers vaccinated with Abdala and their repercussion in the protection of the lactating population.

The SARS-CoV-2 spike RBD-specific IgG antibody response that was observed in the breast milk from COVID-19-recovered mothers is a guarantee of their protection against the virus, based on what was asserted by Feng et al. [27] who considered the RBD-IgG as the essential indicator of immune protection against SARS-CoV-2 infection. Sterlin and colleagues [28] stated that SARS-CoV-2-specific IgA antibodies develop earlier than IgG during the initial stages of the disease, results that could agree with ours. Other authors have exposed that anti-SARS-CoV-2 spike RBD-specific IgA antibodies in breast milk can be detected up to at least 10 months after COVID-19 infection [17].

The sVNT found functional antibodies that blocked the interaction between the SARS-CoV-2 receptor binding domain and the recombinant ACE2 receptor. Since the first 5 weeks after vaccination with Abdala, neutralizing antibodies were identified in the 80% of breast milk samples. This finding confirms that the Abdala vaccine achieves its main objective: the induction of anti-RBD antibodies that interfere with the first step of COVID-19 infection. Hernández-Bernal and colleagues [3] had previously demonstrated the presence of neutralizing antibodies, against live virus, in serum samples from subjects vaccinated with Abdala, included in a phase 1-2, randomized, double-blind, placebo-controlled trial.

Similar percentage of breast milk samples with potentially neutralizing antibodies was found by Pérez et al. [29], 83.3% of samples just a month after vaccination, using a commercial sVNT, in breast milk from mothers who received BNT162b2 (Pfizer) or mRNA-1273 (Moderna) vaccines. Furthermore, they reported neutralization activity in 70.4% and 25.0% of breast milk samples at 3- and 6-months post-vaccination, respectively.

The higher neutralizing titers of 16 breast milk samples signify a potentially robust inhibitory response to the RBD-ACE-2 reaction. Although there were no appreciable differences in the age of the study participants, it should be noted that the participants who had these highest neutralizing antibody titers were below the 24 years old. This could be evidence of the role of age in the strength of the immune response against SARS-CoV-2.

In the COVID-19-recovered mothers, the percentage of positive sVNT samples (80%) was the same as that found 5 weeks after the administration of Abdala vaccine. Some studies have shown detectable neutralizing antibodies 6 months after the onset of COVID-19 symptoms [30,31]. The chronic diseases affect most of the group of COVID-19-recovered women included in the present study, but the hypothesis that the immune response in this group is not as strong as in lactating women with better health
condition was rejected again, according to our results: the titers of neutralizing antibodies per sample were similar between mothers fully vaccinated with Abdala and COVID-19-recovered mothers.

The similarity between the neutralizing capacity of the antibodies generated by SARS-CoV-2 infection and the antibodies induced after vaccination with Abdala gives a special value to this Cuban vaccine against COVID-19: it could provide analogous protection to that conferred by SARS-CoV-2 infection. Our results and those published by Hernández-Bernal et al. [3] support the link between neutralizing antibody levels and evidence-based clinical protection, given by the high levels of defense against COVID-19 and the reduction of disease severity that have been achieved in the Cuban population after the vaccination with Abdala. The sequence of the RBD antigen of Abdala was identical to that of the SARS-CoV-2 Wuhan-Hu-1 strain, and it is expected that this vaccine may have reduced its efficacy against the new variants of the SARS-CoV-2 virus. But the decline in vaccine efficacy is not directly associated with the decrease of neutralizing activity. Some authors have discussed that a neutralization threshold is not yet well established below which vaccines no longer protect [2,32]. Vaccines can generate high concentrations of neutralizing antibodies, which although their activity may be reduced, is likely to have a minor effect on the vaccine efficacy [2].

Hence, SARS-CoV-2 spike RBD-specific IgA antibodies present in breast milk are expected to perform its neutralization function in the mucosa, which implies a potential passive protection received by breastfed infants from their mothers vaccinated with Abdala. Undoubtedly, an important defense for infants in whose vaccination schedule Abdala is not still included.

In conclusion, this work is the first report about the anti-SARS-CoV-2 immune response in breast milk after the administration of the complete vaccination schedule of a COVID-19 protein subunit vaccine, the Cuban vaccine Abdala. High levels of SARS-CoV-2 spike RBD-specific IgA antibodies remain until the ninth weeks after vaccination, and the IgG isotype of these antibodies increases during that time. Potentially neutralizing antibodies are present in most of the evaluated breast milk samples. Neutralizing antibody titers are similar from those found in breast milk samples collected from mothers recovered from COVID-19, which signifies that vaccination with Abdala could provide comparable protection to that conferred by SARS-CoV-2 infection, and breastfeeding could have a protective effect against COVID-19 in the unvaccinated and vulnerable population of newborns and infants.

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Author contributions

Maylin Pérez-Bernal: Conceptualization, Data curation, Investigation, Methodology, Visualization, Writing – review & editing. Carlos Hernández: Formal analysis, Methodology, Validation, Writing – review & editing. Rafael Ibarzollín: Resources, Software, Writing – review & editing. Midalis Martínez: Resources. Migdalia Soria: Resources. Magali Delgado: Investigation. Onel Valdivia: Investigation. Dayamí Dorta: Investigation. Andy Domínguez: Investigation. Enrique Pérez: Project administration, Supervision. Yeovsany Cabrera: Conceptualization, Methodology, Project administration, Supervision.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data available statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics statement

Ethical approval was granted by the Institutional Review Board from the General Hospital “Camilo Cienfuegos”, Sancti Spiritus, Cuba, on August 23, 2021.

Informed consent

Written informed consent was acquired from all participants prior to the collection of breast milk.

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