BRIEF REPORT

Quantitative Analysis of Vertical Transmission of Maternal SARS-CoV-2 Antibodies to Neonates and Young Infants Following Immunization During Pregnancy

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Serum antibody levels to SARS-CoV-2 in infants born to mothers who had received 2 doses of the BNT2b2 vaccine during pregnancy correlated positively with increasing gestational age at vaccination ($P < .01$) and negatively with increasing time from vaccination ($P < .01$), with a significant drop in infants aged >60 days ($P = .045$).

Key words. COVID-19; infancy; neonates; pregnancy; vaccination.

The rapidly increasing coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), stimulated international alliances and government efforts to develop vaccines on shortened timelines. Although pregnant women were initially not included in the trials of the mRNA BNT162b2 vaccine, the American College of Obstetricians and Gynecologists supported the vaccination of pregnant women, partially because of the increased risk of COVID-19 complications during pregnancy [1]. Vaccination of Israeli pregnant women during the second or third trimester was initiated in early 2021, following the Ministry of Health's recommendation.

Vaccination during pregnancy is a confirmed strategy to provide passive infantile immunity against infections, such as pertussis [2] and influenza [3]. Growing evidence has confirmed the vertical transfer of maternal anti-spike immunoglobulin G (IgG) against SARS-CoV-2 after natural COVID-19 infection during pregnancy [4, 5]. Completion of 2-dose mRNA COVID-19 vaccination during pregnancy is associated with lower rates of COVID hospitalization in infants younger than 6 months [6].

The presence of SARS-Cov-2 spike antibodies in infant serum and their dynamic changes in the first few months of life are still unclear. We investigate the presence of these antibodies in the sera of infants born to mothers who had been vaccinated during pregnancy.

METHODS

Study Population

The study was conducted in the Emergency and Pediatric Departments at Schneider Children's Medical Center between May 20, 2021, and August 31, 2021. Infants born to mothers who had been vaccinated with the BNT162b2 COVID-19 vaccine during pregnancy were eligible to participate. Following informed consent, residual sera from the infants' blood samples that were taken as part of medical evaluations were collected and stored. Mothers who escorted their infants during the visits were offered to participate as well; following consent, blood was drawn from the mothers and examined for the study. The study was approved by the Committee for Human Studies of the Medical Center.

Quantification of SARS-CoV-2 Antibodies

To differentiate between the immune response to previous vaccination and SARS-CoV-2 infection, serum samples were tested both for binding antibodies to the spike (S) glycoprotein (which arise after vaccination or SARS-CoV-2 infection) and nucleoprotein (N) IgG (which arise only after SARS-CoV-2 infection). Quantitative detection of IgG antibodies to SARS-CoV-2 nucleoprotein was performed by the Elecsys® immunoassay (Roche Diagnostics, Mannheim, Germany), as described [7]. The sensitivity and specificity of this assay are >99% [7]. The cutoff index is defined as <1.0 for non-reactive samples. Quantitative detection of IgG antibodies to SARS-CoV-2 spike protein was performed by the Liaison SARS-CoV-2 S1/S2 IgG test (DiaSorin, Saluggia, Italy), as described [8]. The sensitivity and specificity of this assay are 97.4% and 98.5%, respectively. Samples were considered negative for antibody titers <13 AU/mL.

Statistical Analyses

Statistical analyses were performed using Excel 2016 and Matlab 2021b (MathWorks, MA, USA). Continuous variables were reported as either mean (standard deviation [SD] or 95%
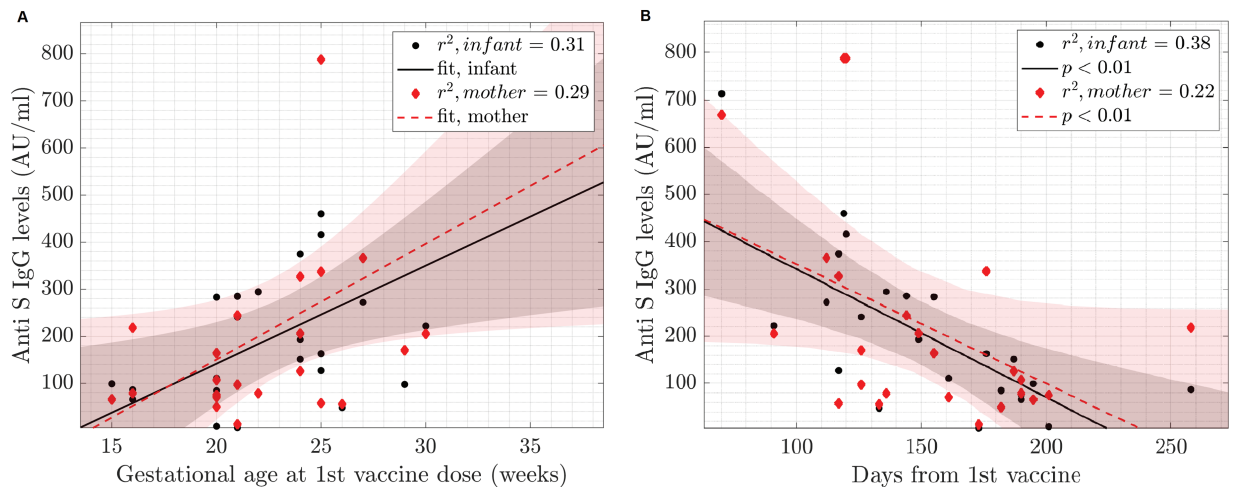
Figure 1. Maternal and infant anti-spike immunoglobulin G (IgG) levels. (A) The correlation with gestational age at the administration of the first vaccine dose. (B) The correlation with the duration from vaccination to blood sampling. The colored area represents the 95% confidence intervals.

confidence interval [95% CI]) or median (interquartile range [IQR]). Correlations were tested using the Pearson correlation coefficient.

RESULTS

Participants and Vaccination Characteristics

The initial study cohort consisted of 37 infants and 27 mothers. Two dyads were excluded from the analysis due to positive anti-N serologies. Two infants were excluded due to the loss of serum specimens. Another 2 dyads were analyzed separately, because the mothers had received only a single dose of the vaccine during pregnancy. The final study group consisted of 31 infants: 22 matched mother-infant dyads, 1 pair of dizygotic twins and their matched mother, and 7 single infants (in cases the mother refused blood sampling). The study flow chart is shown in Supplementary Figure 1.

The mean ± SD age of the infants was 39.8 ± 20.3 days at the time of blood withdrawal. Nineteen infants (61.3%) were males. The mean gestational age at the time of the first vaccine dose was 23 ± 4.6 weeks; the majority of the first vaccines were given in the second trimester (90%). The mean time interval between administration of the first vaccine and blood collection was 145.1 ± 41.7 days. Details are presented in Supplementary Table 1. None of the mothers received a third vaccine dose before enrollment.

SARS-CoV-2 Antibodies in the Infants and Mothers

Of all 31 participating infants, 29 (93.5%) had a positive antibody result (95% CI 0.785-0.99). All 7 infants without a maternal-matched serum sample had high positive tests for S IgG antibodies. Of the 24 infants (including the 2 twins) of participating mothers, 22 presented with positive results for SARS-CoV-2 S IgG antibodies. The remaining 2 infants had low

levels of antibodies, with a relatively low ratio to the matched mother's serum (the infant and maternal S antibody levels were 6 and 13 AU/mL, and 9 and 75 AU/mL, respectively).

The mean levels of SARS S IgG antibodies in infants' and maternal sera were 214.6 AU/mL (95% CI 149.5-279.6) and 198.7 AU/mL (95% CI 117.3-280.0), respectively; the ratio of infant to maternal antibody level was 1.1. Significant higher levels of antibodies were detected in infants younger than 60 days (245.6 [170.4, 320.8 AU/mL]) compared with infants aged 61-90 days (85.7 [15.0, 156.3 AU/mL]; $P = 0.045$). Infants' antibody levels were higher in the 21 infants who were breast-fed (240.4 [156.3, 324.5 AU/mL]) than in the 10 who were exclusively formula-fed (160.6 [70.1, 221.4 AU/mL]), but the difference was not statistically significant ($P = 0.195$). Anti-S (anti Spike protein) levels of the infants were significantly higher with increasing gestational age at the administration of the first vaccine dose ($\rho s = 0.568$, $P < .01$) and were significantly lower with increased duration from the vaccine administration to blood sampling ($\rho s = 0.616$; $P < 0.01$; Figure 1).

Among the 24 infants with matched maternal sera, serum anti-S IgG levels were highly correlated with maternal levels ($\rho s = 0.814$; $P < .001$; Figure 2). Similar results were obtained for sub-group analyses by age category.

Analyzing the infant/mother antibody ratio (infant's anti-SARS-CoV-2 IgG concentration divided by the matched maternal concentration) showed that half of the infants had higher serum antibody levels than their matched mothers. Infant age was negatively correlated with the infant/maternal antibody ratio; hence, the older the infant, the lower the antibody level compared with the mother. Of 13 dyads of mothers and infants aged ≤42 days, 11 (84.6%) had an infant/mother ratio higher than one, which compares with only 1/11 (9.1%) among infants aged 43 to 90 days. The infant/mother antibody ratio by infant age is depicted in Supplementary Figure 2.
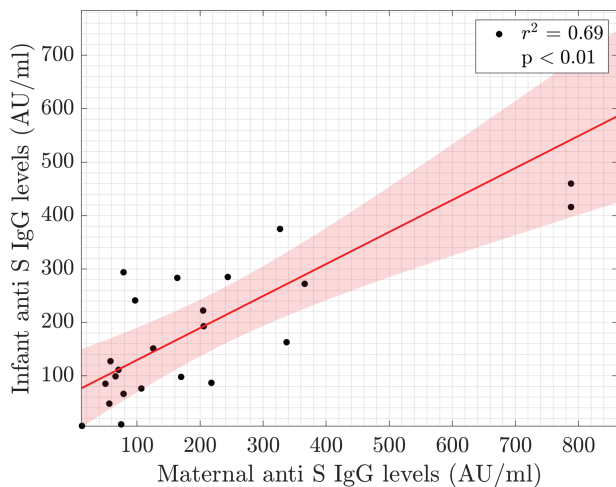
Figure 2. The correlation of serum anti-spike immunoglobulin G (IgG) levels between infants and their matched mothers following vaccination during pregnancy. The dots represent the individual results, and the colored area represents the 95% confidence intervals.

DISCUSSION

This prospective study of maternal and infant serum antibodies to SARS-CoV-2 spike protein following BNT2b2 mRNA vaccination during pregnancy revealed several new findings. First, the vast majority of infants born to these mothers had relatively high levels of serum antibodies against the spike protein; a single vaccine dose during pregnancy did not achieve a sufficient level of antibodies. Antibody presence was previously confirmed in fetal cord blood taken during labor of women who had been vaccinated during pregnancy [9, 10].

We found that postnatal antibody levels were significantly lower in older infants, especially after the age of 60 days. This finding is in concert with the higher antibody levels detected during the first 2 months of life of infants born to mothers vaccinated for pertussis during pregnancy [11]. Our findings are also in line with the immunologic outcome of SARS-CoV-2 infection during pregnancy, in which the vertical transmission of maternal IgG has been confirmed, with antibody persistence up to 6 months [4]. Comparing the serology of infants born to COVID-19-vaccinated mothers and those born to mothers following SARS-CoV-2 infection showed that vaccination resulted in a significantly longer antibody persistence [12]. It should be emphasized that vaccination during pregnancy is primarily intended to protect the pregnant woman.

We found a high correlation between infant and maternal antibody levels ($\rho < 0.001$). This finding aligns with conclusions of studies of other infections [13] and with previous studies that showed a correlation of antibody levels between maternal, cord, and neonatal cord samples on the day of birth [14] Our results showed that in our cohort the infant/maternal ratio of anti-S IgG levels decreased with infant's age; it shifted from >1 (higher in infants) to <1 (higher in mothers) at about age of 40 days.

Antibody levels were lower in older infants, indicating waning immunity.

This study has several limitations. It was conducted in a single center, included a relatively limited number of participants, and examined, for obvious ethical reasons, a single serum antibody level for each infant. We believe that our new findings will stimulate longitudinal large studies to repeat our results and extend them to elucidate the dynamics of SARS-CoV-2 antibodies during infancy. Because of the relatively small numbers, we could not examine the protection from postnatal SARS-CoV-2 infection by maternal immunization during pregnancy nor did we examine the effects of a 3-dose regimen of the vaccine because a third vaccine was not recommended yet when the study was performed.

In conclusion, infants born to mothers who had received 2 doses of the BNT162b2 mRNA vaccine during pregnancy had a relatively high anti-S IgG for up to 3 months. The infant antibody levels correlated positively with maternal antibody levels and with increased gestational age at vaccination. We measured binding antibodies, not neutralizing antibodies; their protection is not proven yet and should be further studied.

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