Vitamin A Supplementation during Pregnancy Enhances Pandemic H1N1 Vaccine Response in Mothers, but Enhancement of Transplacental Antibody Transfer May Depend on When Mothers Are Vaccinated during Pregnancy

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ABSTRACT

Background: In the growing embryo, the vitamin A requirement is tightly regulated. Maternal vitamin A deficiency during pregnancy may alter maternal immune function to accommodate the fetus.

Objective: Our primary objective was to determine the effect of oral vitamin A supplementation (VAS) during pregnancy and until 6 mo postpartum on pandemic H1N1-vaccine responses in mothers and their infants at 6 mo of age.

Methods: In this randomized controlled clinical trial, pregnant women (n = 112) during the second trimester (mean ± SD: 14 ± 1 wk) were assigned to receive either an oral dose of 10,000 IU vitamin A or placebo weekly until 6 mo postpartum. During the third trimester, mothers received a single dose of inactivated pandemic H1N1-influenza vaccine. Hemagglutination-inhibition (HAI) titer was measured in cord, infant, and maternal blood samples. Multivariate regressions with adjustments were used for data analysis.

Results: Seventy-six percent of women had low plasma retinol concentrations (<1.05 μmol/L) in their second trimester. VAS of mothers increased vitamin A concentrations in cord blood by 21.4% and in colostrum by 40.7%. At 6 mo postpartum, women in the vitamin A group had 38.7% higher HAI titers and a higher proportion of HAI titer of ≥1:40 of the cutoff compared with the placebo group. A total of 54.5% of infants had an HAI titer ≥1:40 at 6 mo of age, but there was no difference in HAI titer in infants between groups. Overall, HAI in cord blood did not differ between groups, but in the placebo group, cord blood HAI was negatively associated with maternal “vaccination-to-delivery intervals” (rS = −0.401; P = 0.5), and maternal VAS increased cord blood HAI 6-fold if antenatal immunization was administered ≥10 wk before delivery.

Conclusions: In a community with low vitamin A status, weekly maternal VAS during pregnancy and postpartum increases the breast-milk vitamin A concentration and enhances prenatal H1N1-vaccine responses in mothers, but the benefits of maternal VAS in transplacental antibody transfer may depend on the time of gestation when mothers were vaccinated. This trial was registered at clinicaltrials.gov as NCT00817661. J Nutr 2018;148:1968–1975.

Introduction

Influenza immunization is recommended for all stages of pregnancy. The vaccine appears to be safe for the mothers and fetus (1) and has not been associated with adverse outcomes for the fetus, including spontaneous abortion or fetal death, premature birth, small for gestational age, and congenital malformation (2). Infants of vaccinated mothers have reduced rates of influenza and influenza-related hospitalization in the first 6 mo of life (3). Other nonspecific benefits of maternal immunization include reduction in the risk of stillbirth (2) and reduction in the risk of infants born small for gestational age and with low birth weight (<2500 g) in resource-poor settings (4, 5). Because there is no licensed influenza vaccine for infants aged <6 mo, the strategy of maternal prenatal
influenza immunization can provide effective passive protection to neonates during early life when they are more vulnerable to influenza infection than older infants (6, 7). Our group conducted the first prospective randomized controlled antenatal influenza immunization trial in Bangladesh and found a 63% reduction in laboratory-confirmed influenza illness episodes in infants (5, 8). The present study was conducted in a population in whom the prevalence of vitamin A deficiency among pregnant women is very high. More than half of the pregnant women in Bangladesh consume less vitamin A than the RDA and have low vitamin A status (serum retinol <1.05 µmol/L) (9, 10). There is conclusive evidence that suggests that several aspects of both innate and adaptive immunity are compromised by clinical and subclinical vitamin A deficiency (11–14). Because of the potential risk of teratogenicity, the WHO does not recommend high-dose vitamin A supplementation (VAS) of pregnant women as part of routine antenatal care (15); however, vitamin A deficiency during pregnancy may alter the regulation of maternal immune function to accommodate the growing fetus. Human placenta expresses different types of intracellular-binding proteins for vitamin A metabolites (16). During early organogenesis, the vitamin A content of the placenta is 8-fold higher than the embryonic content, whereas at the end of gestation, the embryonic content is 4-fold higher than the placental content (17). Thus, the amount of vitamin A provided to the fetus is tightly controlled and is usually maintained within an adequate range until maternal stores are depleted (18). Placental transfer of antibody from mother to infant is crucial to sustaining antibody-mediated immunity in the first few months of life. Reduced placental transfer, or low maternal antibody, may result in suboptimal infant antibody concentrations and can increase vulnerability to infection (19). The clinical trial that is reported herein is the first to investigate whether maternal vitamin A supplementation at the recommended level of intake during pregnancy (20) is associated with maternal and infant immune responses to influenza vaccine (clinicaltrials.gov: NCT00817661).

Methods

Study site

This study was carried out in 2010 at the International Center for Diarrheal Disease Research, Bangladesh (icddr,b), in collaboration with Azimpur Maternity Clinic in Dhaka, Bangladesh. The research protocol was approved by the Institutional Review Board of icddr,b. Written informed consent was obtained from all participating mothers after an explanation of the study in the local language.

Study subjects

Mothers visiting the maternity clinic during their first trimester were approached to participate in the study. The inclusion criteria were as follows: pregnant mothers at 11–14 weeks of gestation. The exclusion criteria were residence outside of the clinic catchment area, history of abortion, congenital anomaly, and any previous receipt of influenza vaccine.

Sample size

The sample size was calculated on the basis of the expected increase in serum retinol concentration in response to weekly low-dose VAS during pregnancy compared with the placebo group (21). Because an increase in serum retinol has been associated with significant improvement in dark adaptation, a functional indicator of vitamin A status (21), other functional variables such as antenatal vaccine responses and passive vaccine-antibody transfer to the fetus may also be improved in response to weekly low-dose VAS during pregnancy and the postpartum period.

Primary and secondary outcomes

Our primary outcomes were influenza vaccine responses in mothers and passive antibody transfer to the fetus, as indicated by hemagglutination-inhibition (HAI) titer in infants at 6 mo of age. Our secondary outcomes were vitamin A status in mothers and their infants on the basis of serum retinol concentrations.

Vitamin A and placebo capsules

Unfortified corn oil was used as a diluent to prepare capsules containing 10,000 IU vitamin A from high-dose supplements provided to the mothers after delivery in the national program. Placebo capsules contained an equal volume of corn oil only. The appearance of vitamin A and placebo solutions was similar. A third person unrelated to the study prepared 2 different color-coded capsules; one capsule contained 10,000 IU vitamin A and the other contained 0 IU vitamin A as corn oil. Simple randomization was used for supplementation.

Supplemental Table 1 and Supplemental Figure 1 are available from the “Supplementary data” link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn.

Supplemental Table 1

<table>
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<tr>
<th>Not eligible (n = 27)</th>
<th>Eligible (n = 169)</th>
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</thead>
<tbody>
<tr>
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<td>Lives out of area (16)</td>
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<tr>
<td>Diabetes (2)</td>
<td>Diabetes (2)</td>
</tr>
<tr>
<td>Abortion history (9)</td>
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<td>Refused (n = 57)</td>
<td>Refused (n = 57)</td>
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<td>Infant blood (22)</td>
<td>Infant blood (22)</td>
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<tr>
<td>Vaccination (11)</td>
<td>Vaccination (11)</td>
</tr>
<tr>
<td>Not interested (24)</td>
<td>Not interested (24)</td>
</tr>
<tr>
<td>Enrolled and randomly assigned at 11–14 weeks of gestation (n = 112)</td>
<td>Enrolled and randomly assigned at 11–14 weeks of gestation (n = 112)</td>
</tr>
<tr>
<td>Placebo capsule /wk (n = 56)</td>
<td>Placebo capsule /wk (n = 56)</td>
</tr>
<tr>
<td>Moved out (6) Withdrawn (2) Miscarriage (2)</td>
<td>Moved out (6) Withdrawn (2) Miscarriage (2)</td>
</tr>
<tr>
<td>H1N1 vaccination at 3rd trimester</td>
<td>H1N1 vaccination at 3rd trimester</td>
</tr>
<tr>
<td>Live birth (n = 46)</td>
<td>Live birth (n = 46)</td>
</tr>
<tr>
<td>Study completed (n = 37)</td>
<td>Study completed (n = 37)</td>
</tr>
<tr>
<td>Obtaining infant blood, mother blood &amp; breast milk at 6 mo</td>
<td>Obtaining infant blood, mother blood &amp; breast milk at 6 mo</td>
</tr>
</tbody>
</table>

Figure 1

Mother-infant pairs in the matched H1N1 vaccine response trial. *According to the national health policy, all mothers received one single high-dose vitamin A (200,000 IU) supplement after delivery. VA, vitamin A.

Support

The study was designed as a randomized, placebo-controlled, double-masked trial. Women (n = 112) were randomly assigned to either the vitamin A or placebo group at enrollment. The overall study participant flow is outlined in Figure 1.

Maternal vitamin A supplementation and H1N1 responses 1969
In an exploratory analysis, we investigated the effects of maternal VAS and the time of vaccination in relation to delivery, “vaccination-to-delivery interval,” on passive antibody transfer to the fetus.

**Study procedures.** Pregnant women received weekly capsules containing either 10,000 IU vitamin A or placebo (corn oil) from the beginning of the second trimester until 6 mo postpartum. Fieldworkers delivered the supplements to mothers and directly observed their consumption. At 26–30 weeks of gestation, all mothers received pandemic influenza vaccine A/California/7/2009 (H1N1) virus–like strain [X-179A; Pandemrix, lot AFLSA203AA; GlaxoSmithKline as recommended by the WHO (obtained from the Institute of Public Health, Dhaka, Bangladesh)]. The study physician administered the vaccine intramuscularly and monitored the mothers for any persistent side effects, such as a headache, muscle aches, malaise, fever, or nausea. These side effects usually disappeared within 1–2 d without treatment. As part of the national health policy, all mothers received one single high dose of vitamin A (200,000 IU) at 6–7 wk postpartum. Fieldworkers ensured that infants received all required vaccines according to the recommendations in the national program.

**Blood collection.** A venous blood sample (5.0 mL) was obtained from mothers in the second trimester before starting supplementation. Cord blood (5.0 mL) was collected at birth, and colostrum (5.0 mL) was also collected. Breast milk (5.0 mL) and venous blood were collected from mothers (5.0 mL) and their infants (3.0 mL) within 1–2 wk after the end of maternal supplementation at 6 mo postpartum (Figure 1). Plasma and breast-milk samples were stored at −80°C for analysis.

Gestational ages were calculated from ultrasound data if ultrasound assessments were conducted between 7 and 22 weeks of gestation; otherwise, gestational ages were estimated from the date of the first day of the mother’s last menstrual period.

**Serum and breast-milk retinol concentrations.** Vitamin A concentrations in plasma and breast-milk samples were measured by the HPLC method (12) at icddr, b.

**HAI assay**

HAI assays were performed according to the standard WHO protocol (22). Hemagglutination was measured by the viral agglutination of 0.5% (vol:vol) RBCs from chickens. Serum samples were incubated overnight at 37°C with 4 parts receptor-destroying enzyme to destroy nonspecific inhibitors of hemagglutination. The reactions were stopped by denaturing the enzyme at 56°C for 30 min. Receptor-destroying enzyme-treated serum samples were 2-fold serially diluted in 96-well V-bottom microtiter plates (Nunc), and an equal volume of A(H1N1)pdm09 virus (A/California/7/2009 NYMC X-179A; lot 1213H1AG; catalog no. FR-187; The 2012–2013 WHO Influenza Vaccine Specifications) in PBS was added to each well. The viral growth was assessed after 48 h by determining the presence of hemagglutination. The reactions were stopped by heat at 56°C for 30 min. Both untreated and receptor-destroying enzyme-treated serum samples were included in the assay. Hemagglutination units were added. HAI titers were determined by the reciprocal dilution of the last well, which contained nonagglutinated RBCs.

**Data analysis**

Statistical analyses were conducted with the use of STATA 13 (StataCorp LLC). Distributions of study variables were analyzed to test assumptions of normality and equal variance. Data were log- and/or square root–transformed to normalize distributions as needed. t-Tests or Mann-Whitney Rank Sum tests were used to compare between-group responses. Chi-square tests were used for categorical data analysis, and Spearman’s ρ correlations were carried out to determine the association between 2 variables. Multivariate regression models were used to estimate the treatment effect, controlling for other variables including HAI titer at the second trimester, before vaccination. The selection of variables in the final model was based on the association (P < 0.05) with outcome variables (e.g., vitamin A status and HAI responses). The interval between maternal H1N1 vaccination and delivery can influence HAI titer in cord blood (23) so that antibody transfer to the cord blood can reach >90% of the peak at 10 wk after vaccination in a group of women, presumably with sufficient vitamin A status. In the exploratory analysis, mothers in both groups were stratified on the basis of time between vaccination and delivery [vitamin A <10 wk (n = 19), vitamin A ≥10 wk (n = 20), placebo <10 wk (n = 17), placebo ≥10 wk (n = 20)]. Two-factor ANOVA followed by Holm-Sidak post hoc comparisons were used if there were significant interactions between study groups and the vaccination-to-delivery interval. The overall significance level of these tests was set at P < 0.05.

**Results**

**Enrollment**

After screening, 112 pregnant women were enrolled (Figure 1). Eleven of the women were lost to follow-up because the family moved out of the area (5 in the vitamin A group and 6 in the placebo group). During the pregnancy period, there were 3 miscarriages before the maternal influenza vaccination was administered (1 in the vitamin A group and 2 in the placebo group). The median time interval for “vaccination-to-delivery” in the vitamin A group was 10.0 wk (range: 3.0–13.4 wk) and that for the placebo group was 10.1 wk (range: 4.5–14.0 wk). There were 93 live births (47 in the vitamin A group and 46 in the placebo group); among these, 4 women had preterm deliveries at 30–32 weeks of gestation and were excluded. Infants with an estimated gestational age >36 wk were included in the analysis. Cord blood samples were not collected from infants who were delivered at home (12 in the vitamin A group and 9 in the placebo group). After delivery, 13 mothers were lost to follow-up because the family moved out of the area or the mother decided not to continue participating in the trial for personal reasons. None of the mothers reported night blindness during the study period.

There were no significant differences between the vitamin A and placebo groups in the characteristics of the mothers or infants who completed the study (Table 1). Three infants were born with low birth weight (<2500 g), 2 in the vitamin A group and 1 in the placebo group. According to the national health policy, all mothers at 6–7 wk postpartum received a single 200,000-IU vitamin A supplement (Figure 1). At the time of blood collection at 6 mo postpartum, infants were free from infection. All infants were breastfed, but exclusive breastfeeding was 18.4% and others were “predominantly breastfed.” More than 95% of mothers in both groups complied with the capsule intake.

**Table 1** Characteristics of the 2 study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>VA</th>
<th>PL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, y</td>
<td>24.1 ± 4.48</td>
<td>23.4 ± 4.13</td>
<td>0.46</td>
</tr>
<tr>
<td>Week of pregnancy at enrollment, wk</td>
<td>13.5 ± 0.80</td>
<td>13.7 ± 0.78</td>
<td>0.48</td>
</tr>
<tr>
<td>Gestational age, wk</td>
<td>38.1 ± 1.32</td>
<td>38.9 ± 1.33</td>
<td>0.47</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>2.95 ± 0.39</td>
<td>2.91 ± 0.30</td>
<td>0.58</td>
</tr>
<tr>
<td>Male sex (infants), %</td>
<td>46</td>
<td>55</td>
<td>0.66</td>
</tr>
<tr>
<td>Gestational age at H1N1 vaccination, wk</td>
<td>29.4 ± 2.89</td>
<td>29.1 ± 2.38</td>
<td>0.92</td>
</tr>
<tr>
<td>Vaccination-to-delivery interval, wk</td>
<td>9.67 ± 2.57</td>
<td>9.72 ± 2.31</td>
<td>0.92</td>
</tr>
<tr>
<td>Birth by cesarean delivery, %</td>
<td>36</td>
<td>35</td>
<td>0.87</td>
</tr>
<tr>
<td>Place of birth (clinic), %</td>
<td>69</td>
<td>76</td>
<td>0.71</td>
</tr>
<tr>
<td>Exclusive breastfeeding at 6 mo, %</td>
<td>18</td>
<td>22</td>
<td>0.77</td>
</tr>
</tbody>
</table>

1Values are means ± SDs unless otherwise indicated. Mothers were supplemented weekly either with 10,000 IU vitamin A (n = 38) or placebo (n = 37) and received an H1N1 vaccine at the third trimester. PL, placebo; VA, vitamin A.
Values were log-transformed.

**TABLE 2**

Multivariate regression model for colostrum vitamin A status and HAI responses in the PL and vitamin A groups

<table>
<thead>
<tr>
<th>Cord blood HAI</th>
<th>Mothers’ HAI at 6 mo</th>
<th>Infants’ HAI at 6 mo</th>
<th>Colostrum VA</th>
</tr>
</thead>
<tbody>
<tr>
<td>β (SE)</td>
<td>P</td>
<td>β (SE)</td>
<td>P</td>
</tr>
<tr>
<td>Group (1 = VA, 0 = PL)</td>
<td>-0.010 (0.110)</td>
<td>0.95</td>
<td>0.204 (0.088)</td>
</tr>
<tr>
<td>Mothers’ HAI at enrollment&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.350 (0.097)</td>
<td>&lt;0.01</td>
<td>0.529 (0.077)</td>
</tr>
<tr>
<td>Mode of delivery (1 = normal, 0 = cesarean)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>1</sup>HAI, hemagglutination-inhibition; PL, placebo; VA, vitamin A.

<sup>2</sup>Values were log-transformed.

Impact of maternal VAS on HAI-H1N1 responses

Maternal H1N1 vaccination during the third trimester significantly improved HAI-antibody titer in mothers of both groups at 6 mo postpartum (Figure 2). We found that 34.2% of mothers had an HAI titer of ≥1:40 at enrollment during the second trimester, although they had no history of influenza vaccination. The baseline HAI titer was significantly correlated with postvaccination HAI titer measurements in cord blood and in mothers and infants at 6 mo postvaccination (Supplemental Table 1). Statistical adjustment of the baseline titer showed significantly higher HAI titers in mothers in the vitamin A group at 6 mo postpartum (Table 2). Similarly, all of the mothers in the vitamin A group and 86.5% of mothers in the placebo group had HAI titers of ≥1:40 at 6 mo postpartum, and the differences between groups were significant (P = 0.05) (Supplemental Figure 1).

Maternal VAS did not increase HAI-antibody titer in cord blood (Figure 2, Table 2, Supplemental Figure 1). However, there were significant interactions between study groups and the time interval between maternal vaccination and delivery in predicting both HAI titer and total IgG concentration in cord blood. The vaccination-to-delivery interval had no effect on cord blood HAI titer in the vitamin A group, but in placebo group women in the <10-wk interval showed significantly higher HAI titers (Figure 3A) and total IgG in cord blood (Figure 3B). Interestingly, in the ≥10-wk interval women in the vitamin A group showed an ~6-fold higher cord blood HAI titer compared with that in the placebo group (P < 0.01) (Figure 3A). A similar trend of higher total IgG was also observed (Figure 3B). In agreement with these results, there were significant negative associations between cord blood HAI and the vaccination-to-delivery interval in the placebo group (r<sub>S</sub> = −0.401, P = 0.05) but not in the vitamin A group (r<sub>S</sub> = 0.188, P = 0.37). Significant positive associations between cord blood total IgG and cord blood HAI antibody in the vitamin A group (Table 3) indicated the broader enhancing effects of maternal VAS on transplacental IgG transfer.

Infants of both groups at 6 mo of age had comparable concentrations of HAI antibodies (Figure 2, Table 2), and there were no differences in the proportion of infants who were positive for discrete HAI cutoffs (Supplemental Figure 1). Overall, we found that 54.3% of infants had an HAI titer of ≥1:40. There were no significant interactions between study groups and the vaccination-to-delivery interval in infants’ HAI titer. However, within the placebo group, vaccination at <10 wk of delivery showed significantly higher HAI titers in infants (Figure 3C), similar to the cord blood response. In contrast, high HAI responses in cord blood, as observed among mothers of the vitamin A group who received vaccine at ≥10 wk of delivery (Figure 3A), did not persist in their infants’ HAI titers at 6 mo of age (Figure 3C). However, in both groups, there were significant positive associations between cord blood and infants’ HAI titers (Table 3).

**Mothers’ and infants’ vitamin A status**

Mothers in the vitamin A and placebo groups had similar serum retinol concentrations at enrollment during the second trimester. However, low vitamin A status was highly prevalent in this community (Table 4); 76.4% of pregnant women in their second trimester had low vitamin A status (serum retinol <1.05 μmol/L), with approximately one-fifth (19.7%) classified as deficient (serum retinol <0.70 μmol/L). Weekly 10,000-IU VAS significantly improved cord blood retinol by 21.4% (Figure 4A) and decreased the proportion of women with low serum retinol (<0.70 μmol/L) in cord blood by 20.4% compared with placebo (Table 4). Cord blood retinol was negatively associated with gestational age in the placebo group but not in the vitamin A group (Figure 4B). However, weekly antenatal and postnatal VAS in this study did not enhance plasma vitamin A concentration in mothers or their infants at 6 mo postpartum (Figure 4A). This could be a result of supplementing all mothers in both groups with a single dose of 200,000-IU vitamin A at 6–7 wk postpartum as part of national policy. Maternal supplementation significantly improved vitamin A concentrations in colostrum and breast milk at 6 mo postpartum (Figure 4C) and significantly decreased the proportion of deficient (<0.70 μmol/L) status (24) in breast milk at 6 mo (Table 4). Mothers who underwent cesarean...
delivery had significantly lower colostrum vitamin A \( (P = 0.05) \), and HAI-antibody titers of mothers at enrollment were positively associated with colostrum vitamin A (Supplemental Table 1). Adjusting for these covariates also showed that colostrum vitamin A was higher in the vitamin A-supplemented mothers (Table 2).

**Discussion**

This study shows that low-dose weekly VAS during pregnancy and for 6 mo postpartum significantly increased H1N1 HAI-antibody responses in mothers at 6 mo postpartum who received pandemic H1N1 vaccines in their third trimester. The HAI titer is the primary correlate of protection (25) in which an antibody directed to the strain-specific Hemagglutinin antigen in response to influenza vaccination inhibits the hemagglutination and allows quantification of these antibodies to determine the antigenic response of influenza strains (26). Vitamin A is known to be an immune modulator, and evidence suggests a regulatory role of vitamin A in controlling immune cell development and differentiation in response to various stimuli, including vaccines. VAS at routine infant immunization visits enhances antibody responses to several vaccines (27–29). However, one study reported no association between serum retinol concentration and HAI response in elderly persons (30). Nevertheless, pregnancy is associated with altered hormonal and immunologic changes, including increased estrogen, progesterone, and cortisol concentrations and decreased type 1 to type 2 lymphocytic cytokine responses (31, 32). These changes can directly influence antenatal vaccine responses. VAS of undernourished pregnant women can increase plasma progesterone concentrations (33), and in our previous study, we found that VAS can bias type 2 immune responses to a naïve vaccine (12). The mechanism by which vitamin A regulates hormonal and immune responses during pregnancy is not yet known.

We observed a potential regulatory role of vitamin A in the transplacental transport of maternal antibody in association with gestational age at the time of vaccination. VAS during pregnancy significantly enhanced transplacental antibody transfer if the H1N1 vaccine was provided \( \geq 10 \text{ wk} \) before delivery (Figure 3A). Transplacental transfer of maternal IgG to the growing fetus is not distributed equally throughout the gestational period; in the first trimester, very little IgG is transported (34, 35), it increases to \( \sim 10\% \) of the maternal level in the second trimester, and further increases up to \( \sim 50\% \) in the third trimester (36). Notably, the expansion of fetal IgG concentration between 28 and 41 weeks of gestation is twice that between 17 and 28 weeks of gestation (35). The median gestational age of influenza vaccination in both of our study groups was 29 wk, and we can assume optimal antibody transfer to the fetus. However, cord blood antibody against H1N1 vaccine can appear within 1–2 wk of maternal immunization, but the highest antibody titer in the cord blood appeared when mothers received vaccines between 4 and

**TABLE 3** Spearman’s \( \rho \) correlation between cord blood total IgG and HAI responses in the vitamin A and PL groups

<table>
<thead>
<tr>
<th></th>
<th>VA</th>
<th>PL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cord blood HAI</td>
<td>Infant HAI at 6 mo</td>
<td>Cord blood HAI</td>
</tr>
<tr>
<td>( r_S )</td>
<td>0.413</td>
<td>0.504</td>
</tr>
<tr>
<td>( P )</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>( n )</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Cord blood HAI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( r_S )</td>
<td>0.606</td>
<td>0.749</td>
</tr>
<tr>
<td>( P )</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>( n )</td>
<td>23</td>
<td>19</td>
</tr>
</tbody>
</table>

\( ^1 \text{HAI, hemagglutination-inhibition; PL, placebo; VA, vitamin A.} \)
TABLE 4 Proportion of vitamin A deficiency (<0.70 µmol/L) in the PL and vitamin A groups

<table>
<thead>
<tr>
<th>Group</th>
<th>VA</th>
<th>PL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal plasma at the second trimester</td>
<td>20.5</td>
<td>18.9</td>
<td>0.91</td>
</tr>
<tr>
<td>Cord blood</td>
<td>74.1</td>
<td>92.9</td>
<td>0.07</td>
</tr>
<tr>
<td>Maternal plasma at 6 mo postpartum</td>
<td>2.56</td>
<td>2.70</td>
<td>1.00</td>
</tr>
<tr>
<td>Breast milk at 6 mo postpartum</td>
<td>7.69</td>
<td>32.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Infants’ plasma at 6 mo of age</td>
<td>53.9</td>
<td>64.9</td>
<td>0.45</td>
</tr>
</tbody>
</table>

1 n = 39 (VA); n = 37 (PL). PL, placebo; VA, vitamin A.

<10 wk before delivery (23). These data and the kinetics of transplacental IgG transfer to the growing fetus during gestation were consistent with the mothers in the placebo group, in whom we found a significantly higher HAI titer (as well as total IgG) in cord blood if the mothers received vaccines at <10 wk instead of at ≥10 wk of delivery. Likewise, there was a significant negative association between cord blood HAI and the vaccination-to-delivery interval \( r_s = -0.401, P = 0.05 \) in the placebo group only. Thus, vitamin A has a differential regulation for transplacental antibody transfer. However, there was a 22.5% higher HAI titer in the cord blood samples of the vitamin A–supplemented mothers, which is a notable difference but may be related to an insufficient sample size to detect significance, particularly given the effect of VAS on cord blood HAI in mothers immunized ≥10 wk before delivery. To reach the fetal circulation, maternal IgG must cross many anatomic barriers, including syncytiotrophoblast and cytotrophoblast cell barriers, and then transfer across the villous stroma to make contact at the lumen of fetal endothelial vessels (37).

Several steps in this process are not completely understood. However, studies suggest that the Fc receptor neonatal (FcRn) expressed on syncytiotrophoblast cells plays a central role in the maternofetal delivery of IgG (38). It is not known whether vitamin A controls FcRn expression; yet, significantly higher HAI in the cord blood of the vitamin A-supplemented mothers who received vaccines within ≥10 wk of delivery indicated that vitamin A might enhance FcRn or other receptors at the time of low-level transplacental transfer of antibody. As the interval between vaccination and delivery shortened (e.g., <10 wk), high-level transplacental transfer of antibody (34–36) resulted in comparable cord blood HAI concentrations in the 2 groups. Cord blood total IgG also followed a similar pattern of response (Figure 3B).

At 6 mo of age, HAI titers in infants from both groups of mothers were similar. Overall, 34.5% of infants had HAI titers of ≥1:40 against the A/California/7/2009 (H1N1) compared with 18% HAI titers of ≥1:40 against A/New Caledonia (H1N1) in infants of mothers who received A/New Caledonia (H1N1) vaccines during pregnancy in a previous study (39). Apart from possible differences in vaccine antigens, high (34.2%) prevaccinated maternal HAI in this study, compared with 10% in the previous study (39), might be linked to a higher proportion of responders in infants. In the placebo group, HAI response patterns in relation to the vaccination-to-delivery interval were similar in both cord blood (Figure 3A) and infants at 6 mo (Figure 3C), but in the vitamin A group, higher HAI in the cord blood of the mothers who received vaccine at ≥10 wk of delivery did not carry on later in life. Overall, <10% of HAI in cord blood was detected in infants at 6 mo of age, and a significant positive association between cord blood and infant HAI in both groups (Table 3) reflected a gradual decline in the transplacentally acquired maternal antibody.

Low vitamin A status is highly prevalent among pregnant women in this community. The weekly vitamin A dose (10,000 IU) in this study is the acceptable Tolerable Upper Intake Level of vitamin A from the diet during pregnancy and Maternal vitamin A supplementation and H1N1 responses
lactation [Institute of Medicine, 2001 (20)]. However, the RDA for vitamin A has been set at ~2500 IU during pregnancy and ~4300 IU during lactation (20). Nevertheless, 10,000 IU vitamin A is similar to the doses previously used for weekly supplementation in pregnant women (40, 41) and has been shown to be safe. In our study, weekly supplementation improved cord blood but not infant and mother vitamin A concentrations at 6 mo, which might be an effect of a single high-dose (200,000 IU) VAS to all mothers within 6–7 wk postpartum as part of the national policy. However, improved breast-milk vitamin A status at 6 mo postpartum along with significantly reduced low (<0.70 μmol/L) breast-milk retinol concentrations (Table 4) indicate that breast-milk vitamin A status responds more rapidly to the weekly smaller vitamin A dose than to a single megadose.

Cord blood vitamin A was negatively associated with gestational age in the placebo group but not in the vitamin A group, which indicated that VAS during the antenatal period resulted in an optimal supply of vitamin A as gestation progressed, with a higher demand by the growing fetus. In relation to gestational age, there is a triphasic distribution pattern of vitamin A in the cord blood samples of well-nourished women (42), in whom, until 36 weeks of gestation, the vitamin A concentration remained static in cord blood and then increased sharply between 36 and 39 wk (42). Similarly, in our study, we observed a significant positive association ($r = +0.693$) between gestational age (36–39 wk) and cord blood vitamin A among vitamin A–supplemented mothers. Conversely, in the placebo group, this association was negative ($r = -0.342$). These data also indicate that the amount of vitamin A provided to the fetus is tightly controlled and maintained unless maternal stores are depleted (18, 43). However, the progressive decrease in cord blood retinol with gestational age in the placebo group may have other developmental consequences, because there is no de novo fetal synthesis of vitamin A and the developing mammalian embryo is entirely dependent on the maternal circulation for its vitamin A supply (44).

The strength of our current research is that it is one of the first studies to explore the effects of maternal VAS on clinically relevant vaccine responses in a population in whom low nutritional status is widespread. Considering the high prevalence of low vitamin A status in our study population, it seems that weekly supplementation with 10,000 IU vitamin A might be suboptimal. A limitation of our study is that we did not collect maternal blood around the time of delivery, which limits our understanding of whether HAI in cord blood reflected a differential antibody production in the mothers or differential transplacental transport.

Newborn infants have an immature immune system and are unable to protect against vaccine-preventable infections (45). Transplacental transport of maternal IgG is the principal humoral protection against vaccine-preventable diseases for infants. Because maternal vitamin A stores are the single source of vitamin A for the growing fetus, our results provide further credence to the notion that vitamin A status is an essential regulator of vaccine responses in populations in whom low vitamin A status is widespread.

Acknowledgments

We acknowledge the help of Md Sirajul Islam and Chinmoy K Das for overseeing the study at the Maternal and Child Health Training Institute, Azimpur, Dhaka. The authors’ responsibilities were as follows—SMA: conceptualized and designed the study, conducted the statistical analysis and interpreted the results, and wrote the manuscript; MJA: performed the laboratory assays and contributed to analyzing the data and writing the first draft of the manuscript; AK, MR, and SI: conducted the laboratory assays and supervised the field research; YK and RR: interpreted the findings and critically reviewed the manuscript; MCS: contributed to conceptualizing and designing the study and critically reviewed the manuscript; and all authors: read and approved the final manuscript.

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