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Effect of withholding breastfeeding on the immune response to a live oral rotavirus vaccine in North Indian infants☆

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ABSTRACT

Interference from transplacental and breast milk antibodies may impede the performance of oral live vaccines. The effect of breastfeeding on the immunogenicity of Rotarix®, a two-dose oral monovalent rotavirus vaccine, was examined in a community-based trial in New Delhi, India. Four hundred mother–infant pairs were randomized into two equal groups. Infants were aged 6–7 weeks at enrollment. Mothers were encouraged to either breastfeed or to withhold breastfeeding during the 30 min prior to and after each vaccine dose was administered. We collected blood specimens from infants at enrollment and 4 weeks after the second vaccine dose. Blood and breast milk specimens were obtained from mothers at baseline and breast milk specimens were collected at the time of the second vaccine dose. Seroconversion was defined as infant serum anti-VP6 IgA antibody level of ≥20 IU/mL 4 weeks after the second vaccine dose *and* a ≥4-fold rise from baseline. There was no difference in the proportion who seroconverted between the two groups (26% vs 27%; $p = 0.92$). The levels of infant serum IgA, maternal serum and breast milk IgA and IgG anti-rotavirus antibodies predicted the anti-rotavirus IgA level in infants at end-study and explained approximately 10% of the variability of the immune response ($r^2 = 0.10$, $p < 0.001$).

In this population, the immune response to Rotarix® was not enhanced by withholding breastfeeding around the time of vaccination. Maternal anti-rotavirus antibodies explained little of the variability in the immune response to the vaccine. Factors other than maternal anti-rotavirus antibodies probably explain why infants in low-and middle-income settings respond poorly to live oral rotavirus vaccines.

1. Introduction

Rotavirus is the leading cause of fatal and severe diarrhea in children [1]. In India, it is responsible for almost 100,000 deaths annually [2]. The WHO has recommended inclusion of rotavirus vaccines in all national immunization programs. Currently there are two licensed rotavirus vaccines available; Rotarix®, GSK Biologicals and RotaTeq®, Merck & Co. Both vaccines have demonstrated high efficacy (>90%) against severe rotavirus diseases and rotavirus associated hospitalization in clinical trials in high- and middle-income countries [3–5]. However, trials of these two vaccines conducted in developing settings in Africa and Asia showed lower efficacy, of approximately 60% [6–9]. Most recently, the indigenously manufactured live, oral 116E monovalent human–bovine vaccine has completed an efficacy trial and is expected to be licensed in India

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soon. The efficacy of the 116E vaccine was 54% [10] which is similar to that of Rotateq® and Rotarix® in these settings.

Other live oral vaccines have also performed poorly in low-income countries as compared to more affluent countries [11]. Current evidence indicates that decreased vaccine performance could be attributed to several factors including child or maternal malnutrition, environmental enteropathy, interference from maternal antibodies and presence of other intestinal infections [11]. Presence of rotavirus antibodies in breast milk and transplacental maternal antibodies is associated with impaired responses to rotavirus vaccines [12–14]. Indian women seem to have higher concentrations of rotavirus neutralizing antibodies in breast milk than women in industrialized countries [15]. In vitro studies of the neutralizing effect of breast milk have suggested that withholding of breastfeeding around the time of rotavirus vaccine administration could improve the immune response to the vaccine [15].

Previous trials of rotavirus vaccines had not shown any difference in the immune response to vaccine regardless of whether breast milk was given or not at the time of vaccine administration. In those trials information on breastfeeding was available, however, breastfeeding was self-reported by mothers and the duration between breastfeeding and vaccination was not adequately assessed [16,17]. A recent study from South Africa reported that abstention from breastfeeding an hour before and after each vaccination had no substantial effect on the immune response to a rotavirus vaccine in HIV-uninfected infants [18].

Without clear evidence, it is difficult to determine whether rotavirus antibodies in breast milk interfere with immune response to oral rotavirus vaccines in infants. It is important to explore this association, as it may help improve the impact of the vaccines. We therefore measured whether withholding breastfeeding around the time of vaccination with Rotarix® would improve its immunogenicity. We also explored the association between maternal serum and breast milk anti-rotavirus antibody concentrations with the immune response in infants after two doses of this vaccine.

2. Material and methods

2.1. Study design and participants

The trial was conducted in typical urban resettlement neighborhoods of South Delhi, India.

Infants aged less than 7 weeks were identified through a household survey. Families of infants aged 6–7 weeks were invited to the study clinic for screening and enrollment. Informed written consent was obtained from all parents and also specifically from the mothers. All enrolled infants received two doses of Rotarix® at 6–7 weeks and at 10–14 weeks of age along with other childhood vaccines (Diphtheria, Pertussis, Tetanus, Haemophilus influenzae B, Hepatitis B and oral Polio).

At the study clinic after consent was obtained, a physician examined the infant. Mother–infant pairs were enrolled if the parents gave consent, infants were aged 6–7 weeks, the weight for age was >−3SD of the WHO child growth standards, and the family had no plans to move out of the study area for the next 4 months. Infants were excluded if they were not breastfed, had already received a rotavirus vaccine, had immunodeficiency disease, chronic enteric disease, and/or any other condition as warranting exclusion by the investigator. Infants were temporarily excluded if they had diarrhea or any illness requiring hospital referral on the day of enrollment.

Eligible infants were either allocated to the group where mothers were requested to withhold breastfeeding for 30 min before and after vaccine administration or to the group where mothers were encouraged to breastfeed their infants around the time of vaccination. There were two separate locations in the study clinic for the two groups to ensure that instructions for breastfeeding were followed by mothers. Clinical coordinators supervised each area. Activities were conducted in the following order: 30 min of withholding or encouraging breastfeeding; administration of Rotarix®; 30 min of withholding or encouraging breastfeeding; administration of other childhood vaccines; observation for 30 min to assess for immediate adverse events. The study team documented the time breastfeeding started and ended as well as the time when the other vaccines were administered.

2.2. Follow-up activities

Infants were observed for immediate adverse events in the study clinic and referred to the hospital, if required. Families of infants were contacted weekly after each dose of the Rotarix® to ascertain presence of signs and symptoms of any illness requiring hospital referral including intussusception, or other serious adverse events. Minor illnesses not requiring hospital referral were managed by the study physician. Serious adverse events were reported to the relevant Ethics Committees.

2.3. Randomization and blinding

The randomization list was generated by a statistician independent of the study team in Stata 11 (StataCorp LP, TX, USA). Eligible infants were randomly allocated to either group through serially numbered opaque sealed envelopes with the group allocation written inside the envelope. The laboratory assessing the immune responses was blinded to the group allocation.

2.4. Assessment of immune response

At enrollment, blood and breast milk specimens were obtained from mothers and blood and stool specimens were obtained from the infants. At the time of the second dose of Rotarix®, a breast milk specimen was obtained from the mother. Four weeks after the second dose of Rotarix®, blood specimen was obtained from each infant.

2.5. Laboratory methods

The specimens were tested at the Wellcome Trust Research Laboratory at Christian Medical College, Vellore. The IgA and IgG titers were determined by comparing the optical density values form sample wells with the standard curve based on derived units of IgA arbitrarily assigned to pooled human serum samples, as previously described [19].

2.6. Statistical analyses

Statistical analyses were carried out in Stata 11.0 (StataCorp LP, TX, USA). Descriptive measures of continuous variables were presented as means and standard deviations for symmetrical data, and as medians and interquartile ranges for skewed data. The Spearman rank-order correlation test was used for comparing median values. Seroconversion was defined as infant serum anti-VP6 IgA antibody level of ≥20 IU/mL 4 weeks after the second vaccine dose *and* a ≥4-fold rise from baseline. We measured the effect of the interventions and other exposures on the proportion who seroconverted and on the log-transformed end study antibody levels of the infants. The relationship between maternal and child antibodies and these outcomes were examined in crude and multivariate logistic and linear regression models. In these models, we initially included variables that were significant on a 0.05 level (from the crude models), we kept those that remained significant and added the other exposure variables one at a time and retained significant variables for the

final model. The ratio between proportions and its corresponding confidence interval was calculated using the binreg command in stata.

2.7. Ethical considerations

Ethical clearance was obtained from Society for Applied Studies, Ethics Review Committee, Christian Medical College, Institutional Ethics Committee and South-East Regional Ethical Committee of Norway. This study was conducted in compliance with the protocol, Good Clinical Practices and other relevant regulatory guidelines.

3. Results

Of the 533 infants screened for eligibility, 400 were enrolled and randomized into two equal groups. All infants received the first dose of Rotarix® and 391 received both doses; four families moved out of the study area and five refused the second dose (Fig. 1).

Both baseline and end study blood specimen were available for 388 infants.

The baseline characteristics were comparable between the groups (Table 1). The proportion of infants being exclusively breastfed was high overall; 75% in the group that was requested to withhold breastfeeding around the time of vaccination and 80% in the group that was encouraged to breastfeed. The mean (SD) age of infants at the time of vaccination was 6.9 (0.56) and 11.2 (0.62) months for the first and second doses, respectively.

The infant and maternal anti-rotavirus antibody levels in the serum and breast milk were similar between the two groups (Table 2). All except one mother in the group that was withholding breastfeeding adhered to the instructions. Infants in the group withholding breastfeeding were not breastfed for a mean (SD) duration of 49 (11.1) and 46 (10.9) min after receiving the first and second doses of Rotarix®, respectively.

The proportions of infants who seroconverted at study end were similar in the two groups; 26% of infants in the group where breastfeeding was withheld and 27% in the group where infants were breastfed ($p = 0.920$) (Table 3). The ratio of the proportion that seroconverted in the two groups was 0.98 (95% CI 0.70, 1.38). The

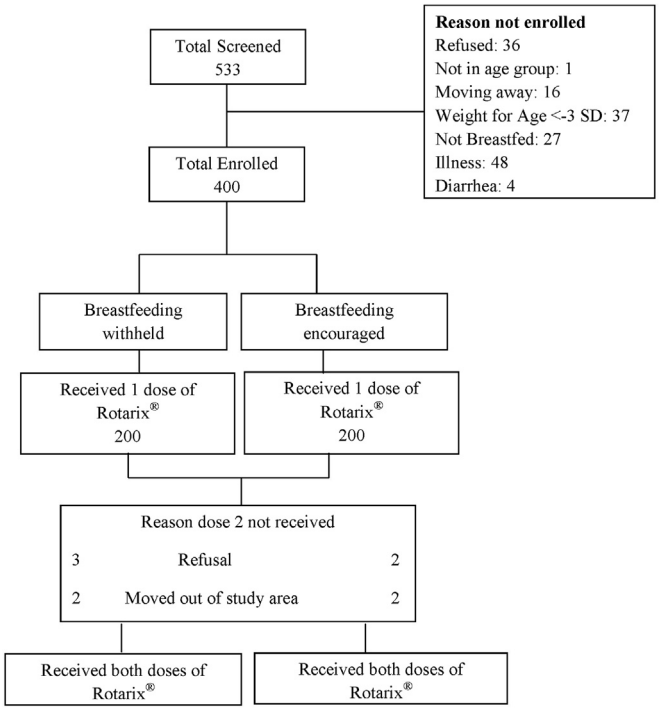

Fig. 1. Trial profile.

maternal serum IgA and IgG at baseline and breast milk IgA and IgG were also significantly associated with the immune response (Table 4). While the infant baseline antibody level was positively associated, maternal antibodies were negatively associated with the immune response. The adjusted model, including infant baseline serum IgA, breast milk IgA and breast milk IgG confirmed these associations (Table 4).

The odds (95% CI) of seroconversion showed similar results with higher odds of seroconversion with increasing levels of infant

Table 1
Baseline characteristics of participants in the two study groups.

	Breastfeeding withheld ($n = 200$)	Breastfeeding encouraged ($n = 200$)
Infant characteristics		
Age at enrollment (days)	48 (4.0)	49 (3.8)
Mean (SD)		
Birth weight[a] (kg)	2.8 (0.4)	2.8 (0.5)
Mean (SD)		
Weight at screening (kg)	4.4 (0.6)	4.4 (0.5)
Mean (SD)		
Sex		
Girls	97 (48.5)	95 (47.5)
Exclusively breastfed	150 (75.0)	160 (80.0)
Socioeconomic characteristics		
Home birth	61 (30.5)	52 (26.0)
Type of family		
Nuclear	112 (56.0)	115 (57.5)
Joint	88 (44.0)	85 (42.5)
Number of siblings	0.95 (0.96)	1.1 (1.1)
Mean (SD)		
Maternal age (years)	24.4 (3.5)	24.8 (3.9)
Mean (SD)		
Mothers who have not attended school	48 (24.0)	45 (22.5)
Fathers who have not attended school	22 (11.0)	22 (11.0)
Family who own color TV, cooler, or scooter	182 (91.0)	179 (89.5)
Annual family income, kRupees median/inter quartile range	84 (60, 120)	84 (72, 120)

All are *n* (%) except when specified otherwise.

[a] Reliable information on birth weight was available for 137 (68.5%) infants in the withholding of breastfeeding group and 143 (71.5%) infants in the group encouraged to breastfeed.

Table 2
Infant and maternal anti-rotavirus antibody concentrations in breastfeeding encouraged and withheld infants (log IU).

	Breastfeeding withheld Median (IQR)	Breastfeeding encouraged Median (IQR)
Infant		
Serum IgA baseline	1.95 (0.33, 5.48)	2.04 (0.59, 4.37)
Serum IgA post dose 2	6.77 (1.95, 35.38)	6.06 (1.72, 29.02)
Maternal		
Serum IgA baseline	210.19 (105.16, 384.56)	164.99 (94.96, 371.05)
Serum IgG baseline	11,562.03 (5402.71, 20,575.12)	9801.49 (4577.05, 23,997.40)
Breast milk IgA baseline	17.63 (9.13, 44.16)	18.23 (9.68, 36.63)
Breast milk IgG baseline	14.26 (0.00, 52.79)	15.85 (0.00, 46.13)
Breast milk IgA at dose 2	17.46 (9.44, 34.81)	17.43 (9.22, 28.94)
Breast milk IgG at dose 2	16.69 (0.00, 46.66)	15.82 (0.00, 53.50)

$p > 0.05$ for all comparisons.

Table 3
Immune responses four weeks after dose 2 of Rotarix® in seronegative infants (serum anti- rotavirus IgA < 20 IU/mL at baseline).

	Breastfeeding withheld $N = 172$	Breastfeeding encouraged $N = 184$	p value
4-Fold rise (seroconversion)[a] *N* %	45 (26.1)	49 (26.6)	0.92
2-Fold rise[b] *N* %	49 (28.5)	51 (27.7)	0.87

[a] Seroconversion was defined as infant serum anti-VP6 IgA antibody level of ≥20 IU/mL 4 weeks after the second vaccine dose and a ≥4-fold rise from baseline.
[b] Defined as infant serum anti-VP6 IgA antibody level of ≥20 IU/mL 4 weeks after the second vaccine dose and a ≥2-fold rise in the anti-VP6 IgA antibody level from baseline.

Table 4
Anti-rotavirus antibody levels in infants and mothers as predictors for anti-rotavirus serum IgA levels 4 weeks after dose 2 of Rotarix® in infants who were seronegative at baseline.

Explanatory variables	Crude estimates			Adjusted estimates[a]	
	Co-efficient (95% CI)	p value	R-Squared	Co-efficient (95% CI)	p value
Infant serum IgA baseline	0.50 (0.30,0.70)	0.000	0.063	0.49 (0.29, 0.69)	<0.001
Maternal					
Baseline mother serum IgA	−0.17 (−0.33, −0.02)	0.031	0.013	–	–
Baseline mother serum IgG	−0.22 (−0.37, −0.06)	0.006	0.020	–	–
Baseline breast milk IgA	−0.23 (−0.38, −0.07)	0.004	0.023	–	–
Baseline breast milk IgG	−0.14 (−0.22, −0.06)	0.001	0.030	–	–
Breast milk IgA at dose 2	−0.26 (−0.42, −0.11)	0.001	0.031	−0.20 (−0.36, −0.05)	0.010
Breast milk IgG at dose 2	−0.15 (−0.23, −0.07)	0.000	0.038	−0.10 (−0.19, −0.02)	0.013

Log transformed anti-rotavirus concentrations.
[a] The adjusted estimates are the results of a multiple linear regression model where $N = 356$, $p < 0.001$ adjusted $r^2 = 0.1047$, only the variables that remained significant were retained in the final model.

serum IgA at baseline and lower odds of seroconversion with increasing levels of maternal antibodies (Table 5).

4. Discussion

We examined the effect of temporarily withholding breastfeeding on the immune response to the live oral rotavirus vaccine Rotarix® in a randomized community trial. Despite excellent compliance to the breastfeeding instructions in the groups where breastfeeding was withheld as well as the group where breastfeeding was encouraged, the proportion of infants who seroconverted was similar in the two groups. These results are similar to those reported from similar studies in South Africa and Pakistan [18,21].

Table 5
Anti-rotavirus antibody concentration in infants and mothers as predictors for seroconversion 4 weeks after dose 2 of Rotarix® in infants seronegative at baseline.

Explanatory variables	Crude estimates	
	Odds ratio (95% CI)	p value
Infant		
Infant serum IgA baseline	1.24 (0.92–1.68)	0.162
Maternal		
Baseline mother serum IgA	0.82 (0.65–1.03)	0.094
Baseline mother serum IgG	0.75 (0.60–0.93)	0.011
Baseline breast milk IgA	0.76 (0.59–0.97)	0.029
Baseline breast milk IgG	0.84 (0.75–0.96)	0.007
Breast milk IgA at dose 2	0.70 (0.54–0.91)	0.007
Breast milk IgG at dose 2	0.87 (0.77–0.98)	0.021

The overall seroconversion rate in our study was low, and factors other than maternal antibodies are likely to be responsible for the poor immunogenicity of the vaccine. A recent Rotarix® trial in south India examined the effect of probiotic and zinc supplementation on the immune response to oral rotavirus and oral poliovirus vaccines. This study reported a 35% seroconversion rate in infants who received the vaccine with probiotic supplementation and 28% in infants who received the vaccine and a placebo. In children who received the vaccine with zinc supplementation the seroconversion rate was 34% compared to 29% in the group receiving the vaccine and a placebo [20]. The infants in the study in south India were of the same age as the infants in our study and in both studies childhood vaccines were given along with Rotarix®. A low seroconversion rate was also seen in Pakistan where they examined the immune response to Rotarix® after withholding breastfeeding for an hour before and after giving the vaccine [21].

An immunogenicity study of Rotarix in India reported a 58.3% seroconversion rate [22]. In this study, the mean age of infants at the time of receiving the first and second doses of the vaccine were 8.7

and 13.4 weeks of age, compared to 6 weeks in our study and in the south Indian study. Also, in this immunogenicity study, an interval of two weeks was maintained between other childhood vaccines and the rotavirus vaccine whereas in our study and in the south Indian study the childhood vaccines were given along with Rotarix. Similar findings were seen with the Indian rotavirus vaccine, ORV 116E, where the immune response in the phase Ia/IIb was much higher than reported in the phase III (90% vs. 40%) [10,23]. In the phase1a/IIb trial, infants were around 8 weeks old at the time of receiving the first dose of the vaccine and there was in interval of two weeks between childhood vaccines and 116E while in the phase III trial, infants were around 6 weeks old and received the childhood vaccines along with the rotavirus vaccine.

It is possible that in both Rotarix and 116E immunogenicity studies the slightly higher age at vaccination and/or maintaining an interval between childhood vaccines and rotavirus vaccines particularly the live oral polio vaccine, may have improved the immune response. It has been described before that co-administration of oral poliovirus vaccine interferes with the immune response to rotavirus vaccines [19,24,25], although polio seroconversion rates are not affected.

Other studies have reported inverse association seen between maternal serum and breast milk IgA and IgG levels of infant IgA levels post dose 2. The 116E vaccine showed an inverse relationship between levels of pre-existing rotavirus IgG and immune response to the vaccine [26]. In our study, preexisitng antibodies at baseline explained only about 10% of the variability in the immune response to the vaccine. Although maternal antibodies impair the immunogenicity, other factors seem to be more important and contribute to the poor immune response.

The protective role of maternal antibodies against rotavirus infection is not clear [13,14,27] although it is suggestive of protection [28,29]. In the previously mentioned study in Pakistan, the seroconversion rate was higher in the group that was breastfed around the time of vaccination, although the difference was not statistically significant. Even if withholding breast milk at the time of vaccination could modify the immune response, the impact would be minimal as the maternal levels explained only a fraction of the variability in the immune responses.

A limitation of our study was that the duration of withholding breastfeeding around the time of vaccination was restricted to 30 min before and after each dose. Studies have shown that half gastric emptying time in infants who were breastfed varied between 47 and 56 min [30–32]. Therefore, half of the breast milk would still be present in the infant's stomach after an hour. Withholding breastfeeding for an hour before and after vaccination would have been appropriate but was not feasible in this study setting. This time interval was also used in the previously mentioned phase 1a/II oral rotavirus vaccine 116E trial which demonstrated good immunogenicity [23]. However, the recent study from south Africa suggest that increasing the window for withholding breastfeeding does not effect the immune response [18]. Additionally, in this study, only infants who were currently breastfed were enrolled. It is possible that maternal antibodies transferred transplacentally or through breast milk to the infant may interfere with the immune response even if mothers withhold breastfeeding around the time of vaccination. The prevalence of exclusive breastfeeding was high at baseline, which is consistent with previous observations in this population [33].

Seroconversion is not a direct indicator of clinical vaccine efficacy but it is nevertheless important as a proxy for vaccine uptake. Mechanisms other than antibody levels may explain the low immune response to rotavirus vaccines. It is worthwhile to explore whether interference with other intestinal infections or micronutrient deficiencies may modify immune responses [34,35].

In conclusion, withholding breastfeeding around the time of vaccination did not improve the immune response to Rotarix® in Indian infants. This suggests that the interference of breast milk with the vaccine 'take' as assumed previously may not be of practical clinical relevance.

Conflict of interest

None of the authors declare any conflict of interest

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