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The induction of breast milk pertussis specific antibodies following gestational tetanus–diphtheria–acellular pertussis vaccination

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ABSTRACT

Background: Center for Disease Control and Prevention recommends vaccination of pregnant women with tetanus–diphtheria–acellular pertussis (Tdap).
Aim: To measure pertussis specific antibodies, total protein and their ratio in breast milk following gestational Tdap vaccination.
Methods: Women who received Tdap after the 20th week of pregnancy were recruited and unvaccinated women served as controls. Breast milk total protein, immunoglobulin A (IgA) to pertussis toxin (PT), filamentous hemagglutinin (FHA) and immunoglobulin G (IgG) to PT, FHA and pertactin (PRN) were measured. To overcome the dilution that occurs in the transition from colostrum to mature breast milk, we calculated pertussis specific antibody to total protein ratio.
Results: Pertussis specific IgA was the predominant pertussis immunoglobulin in the colostrum of Tdap vaccinated women with the geometric mean concentrations (GMCs) of IgA to FHA higher than for IgA to PT, 24.12 ELISA units/milliliter (EU/mL) vs. 8.18 EU/mL, respectively, $p < 0.004$. There were differences between the vaccinated women and controls in the GMCs of IgA to FHA and IgG to PRN in the colostrum, 24.12 EU/mL vs. 6.52 EU/mL, $p = 0.01$ and 2.46 EU/mL vs. <0.6 EU/mL, $p = 0.03$, respectively. The GMCs of total protein showed significant decline over 8 weeks in the vaccinated women and controls, $p < 0.004$. Among vaccinated women, there was significant decline in the GMCs of IgA to PT and FHA over 8 weeks, $p < 0.001$. The geometric mean ratio of IgA to FHA to total protein also declined significantly over 8 weeks in the vaccinated women, $p < 0.01$, demonstrating a true decrease, however, pertussis IgA was measurable at 8 weeks.
Conclusions: Select colostrum pertussis antibody levels were significantly higher among women vaccinated with Tdap during pregnancy compared with unvaccinated women. Among vaccinated women, maximal levels of pertussis specific IgA were in the colostrum but still detected at 8 weeks. Lactation may augment infant's protection against pertussis.

1. Background

There have been increasing reports of high *Bordetella pertussis* (*B. pertussis*) morbidity and mortality in industrialized countries despite widespread acellular vaccine coverage with significant sequelae to young infants [1–5]. Recognizing the vulnerability of unimmunized infants, public health policy makers

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have advocated different strategies in attempt to control *B. pertussis* infection including adolescent vaccination and cocooning [6,7]. More recently, the Center for Disease Control and Prevention (CDC) recommended vaccination of pregnant women with tetanus–diphtheria–acellular pertussis (Tdap) during the third or late second trimester, preferably at 27–36 weeks of gestation, with the anticipation of trans placental humoral protection [8,9].

The current Israeli immunization policy recommends vaccination of infants with acellular pertussis vaccine at ages 2, 4, 6 and 12 months old and pertussis booster doses for children and adolescents at 7–8 years (as of 2005) and 13–14 years (as of 2008), respectively [7]. Moreover, in 2012 the Israeli Ministry of Health recommended vaccination of unvaccinated pregnant women with Tdap after the 20th week. This policy was broadened in 2013 to target all pregnant women during their 27–36 weeks gestation, regardless of their vaccination history.

An additional facet to the humoral protection provided by maternal immunization with Tdap may be the augmentation of protective pertussis antibodies to the lactating infant via breast milk.

Human milk is highly regarded as the ideal nutrient for infants and contains biological molecules that convey considerable immunological benefits including secretory immunoglobulin A (IgA) [10]. Pertussis specific IgA has been demonstrated in the breast milk of postpartum Tdap-vaccinated mothers, 1–2 weeks after vaccination [11].

While the protective attributes of breast milk are established, little is known regarding the transfer of pathogen specific antibodies following vaccination during pregnancy [12]. Specifically, to the best of our knowledge, there is no data on the effect of late second and third trimester Tdap immunization of pregnant women on the levels of pertussis specific antibodies in breast milk [12,13].

The aim of this study is to examine the colostrum and breast milk levels of pertussis specific IgA and immunoglobulin G (IgG), total protein, and the ratio of pertussis specific IgA and IgG to total protein, following vaccination with Tdap during late pregnancy and to evaluate the kinetics of these antibodies prior to the initiation of infant pertussis vaccines.

2. Methods

2.1. Study population

Women with singleton births, gestational age ≥36 weeks who received Tdap after the 20th week of the current pregnancy were recruited. Periparturient women unvaccinated for pertussis during the current pregnancy served as controls. This cohort of women was part of a larger study examining pertussis humoral antibody development following gestational vaccination with Tdap during late pregnancy.

Women with one or more of the following criteria were excluded:

Women who gave birth to infants <2000 g, having an immunologic disorder, receipt of immunoglobulins in the previous year, receipt of immunosuppressive drugs during the current pregnancy or blood products in the previous 3 months before delivery, documented or suspected pertussis infection within the previous 5 years (positive *B. pertussis* culture, positive *B. pertussis* polymerase chain reaction, IgG to pertussis toxin (PT) ≥94 international units (IU)/milliliter (mL) or cough >2 weeks), receipt of a pertussis containing vaccine within 5 years of the current pregnancy; any vaccine besides Tdap within 2 weeks of delivery.

The study was approved by the Medical Center's Institutional Review Board and all participants gave informed consent.

2.2. Vaccine

Pregnant women were vaccinated with Tdap (Boostrix: tetanus toxoid (≥20 IU), diphtheria toxoid (≥20 IU), PT (8 microgram (mcg), filamentous hemagglutinin (FHA) (8 mcg), Pertactin (PRN) (2.5 mcg), Aluminum salts, Formaldehyde, Polysorbate 80, Glycine, Sodium Chloride).

2.3. Study design

During the study period, November 2013–February 2014, periparturient women intending to breast feed were recruited from the obstetrics department at Bnai Zion Medical Center, Haifa, Israel, for an intended 8 week follow-up period.

The participating lactating women were asked to provide a total of 4 breast milk samples. The first sample was collected prior to discharge and designated as colostrum, and the second, third and fourth samples were collected at week 2 (±2 days), week 4 (±2 days) and at week 8 (±2 days) postpartum, respectively. The study participants were instructed to express a 2–5 mL sample and store it in a sterile labeled plastic container that was given to them prior to their hospital discharge. The women were directed to store the expressed human milk in their home refrigerator until pick-up by a study investigator. The sample collection was performed on the same day that the milk was expressed and transferred on ice to the Bnai Zion Medical Center laboratory. Samples were then stored up to one night at 2 °C temperature until processing.

2.4. Breast milk processing

Whole-milk aliquots were processed as recently described by Sara de Schuter et al. to yield maximal levels of IgA to PT [14]. The first centrifugation was at 1000 × *g* for 10 min, and the second centrifugation was at 10,000 × *g* for 30 min. A cotton-coated swab was used to remove the lipid layer fraction after each centrifugation. Aliquots of the aqueous fraction were then transferred to sterile test-tubes and stored at −20 °C in a frost-free freezer until assayed for pertussis specific antibodies. The breast milk cell pellets were discarded.

2.5. Analysis of pertussis specific immunoglobulins

Pertussis-specific IgG (PT, FHA and PRN) and IgA (PT, FHA) were measured by IgG and IgA-specific enzyme-linked immunosorbent assay (ELISA) in breast milk (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany) using a 1:101 dilution as per manufacturer specifications. Results were reported in ELISA Units per mL (EU/mL). The lower levels of PT IgG and IgA detection were 0.2 EU/mL, and 0.7 EU/mL, respectively. The lower limits for FHA IgG and IgA were 1 EU/mL and 0.2 EU/mL. The lower limit of PRN IgG was 0.6 EU/mL.

2.6. Total protein measurement

Total protein levels in breast milk were measured by Bradford assay (SIGMA ALDRICH, MO, USA) using a 1:10 dilution as per manufacturer specifications. Results were multiplied by ten and then were reported as mg/mL.

2.7. Statistics

Demographic and clinical data of the Tdap-vaccinated and the unvaccinated controls were determined by χ^2 and Fisher exact tests where appropriate for dichotomous data and by independent *t*-test or Mann–Whitney U test, in the case of non-normally distributed data. Antibody levels below the limit of detection were assigned the