

OBSTETRICS

Maternal immunization with tetanus–diphtheria–pertussis vaccine: effect on maternal and neonatal serum antibody levels

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OBJECTIVE: We sought to determine whether tetanus–diphtheria–pertussis vaccination (Tdap) in pregnancy provides newborns antibodies against pertussis when compared to mothers who did not receive Tdap.

STUDY DESIGN: Paired maternal and umbilical cord blood samples were collected at the time of delivery and the serum stored at -86°C . For each paired sample of maternal and cord blood, the medical chart and vaccine history was reviewed to determine whether Tdap was received or not.

RESULTS: Newborns born from mothers who received Tdap during pregnancy had significantly higher concentrations of diphtheria antitoxin ($P < .001$), tetanus antitoxin ($P = .004$), and antibodies to pertus-

sis toxin ($P < .001$), filamentous hemagglutinin ($P = .002$), pertactin ($P < .001$), and fimbriae 2/3 ($P < .001$) when compared to newborns from mothers who did not receive Tdap. There was a significant increase in the odds that newborns from mothers who received Tdap during pregnancy have antibodies that may provide protection against diphtheria ($P = .0141$), pertussis toxin ($P < .0001$), and fimbriae 2/3 ($P = .0146$).

CONCLUSION: Administering Tdap during pregnancy increases antibody titers against diphtheria and pertussis antigens. Maternal Tdap may prevent neonatal pertussis infection.

Key words: acellular pertussis antigens vaccine increased neonatal protection, maternal tetanus, reduced diphtheria

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Pertussis (whooping cough) is a respiratory tract infection caused by *Bordetella pertussis*. The disease is most severe in young infants, who have the highest hospitalization and complication rates.^{1,2} Almost all deaths associated with confirmed pertussis infection occur in infants ≤ 6 months of age, most too young to have received their primary series of tetanus toxoid (TT), diphtheria toxoid (DT), and acellular pertussis antigens vaccine.²

The annual incidence of pertussis in the United States has increased 3-fold since 1980, even though immunization rates for young children have been 80%.^{1,3} During 1997 through 2000 in the United States, the highest pertussis attack rate (55.5 cases per 100,000 population) occurred in infants < 1 year of age, which is in contrast to the attack rate of 0.8–5.5 cases per 100,000 population in other age groups.¹ In 2000, each of the 17 pertussis-related deaths reported to the Centers for Disease Control and Prevention occurred in US-born infants who contracted pertussis at ≤ 4 months of age.^{3,4}

In 2005, the Food and Drug Administration licensed the tetanus, reduced diphtheria, and acellular pertussis antigens vaccine (Tdap) for persons 11–64 years of age and in 2006, the Advisory Committee for Immunization Practices (ACIP) recommended Tdap for routine use in adolescents and adults. ACIP recommended the vaccine could be used in pregnancy, but preferred postpartum maternal administration in an effort to immunize the persons who were surrounding the infant.

This report provides the results of active Tdap immunization to 52 pregnant women who were compared to 52 pregnant women who did not receive Tdap during pregnancy. Paired maternal serum and umbilical cord serum were collected for each group. TT and DT antibodies and antibodies to the pertussis antigens pertactin (PRN), pertussis toxin (PT), filamentous hemagglutinin (FHA), and fimbriae (FIM) 2/3 were measured.

MATERIALS AND METHODS

Our subjects were pregnant women attending the University of Louisville Obstetrical Clinic from October 2008 through December 2009. Institutional approval was obtained for use of discarded maternal and umbilical cord blood samples. All patients were encouraged to receive Tdap during the second trimester of pregnancy. However, the exact timing of the administration of Tdap could not be determined as some patients received Tdap prior to pregnancy and some received Tdap at referring clinics. Routinely collected maternal blood

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TABLE 1
Newborn antibody levels stratified whether mothers Tdap

Outcome Antibodies	Mother did not receive Tdap, mean (SEM) n = 52	Mother received Tdap, mean (SEM) n = 52	P value ^a
Diphtheria	0.571 (0.157)	1.970 (0.291)	< .001
Tetanus	4.237 (1.381)	9.015 (0.981)	.004
PT	11.010 (1.796)	28.220 (2.768)	< .001
FHA	26.830 (4.022)	104.15 (21.664)	.002
PRN	24.700 (5.765)	333.01 (56.435)	< .001
FIM 2/3	82.83 (14.585)	1198.99 (189.937)	< .001

FHA, filamentous hemagglutinin; FIM, fimbriae; PRN, pertactin; PT, pertussis toxin; Tdap, tetanus, reduced diphtheria, and acellular pertussis antigens vaccine.

^a Significant at .05 level.

Gall. Effect of maternal immunization with Tdap. *Am J Obstet Gynecol* 2011.

and umbilical cord blood samples were retained, centrifuged to separate the serum, and the serum was frozen at -86°C until analyzed. The medical chart was used as a source for the vaccine history as to whether the patient received Tdap or did not receive Tdap.

Laboratory methods

Vaccine

The Tdap used in this study was manufactured by Sanofi Pasteur (Swiftwater, PA) and contained the following toxoid and antigen concentrations: DT, 2 Lf U; TT, 5 Lf U; PT, 2.5 μg ; FHA, 5.0 μg ; PRN, 3.0 μg ; and FIM, 2/3 5 μg .

Antibody quantitation

DT, TT, PT, FHA, PRN, and FIM antibody levels were measured as previously

described.⁵ Briefly, antigen (a gift from Sanofi Pasteur) was absorbed onto polystyrene microtiter plates. Sera, assayed at dilutions ranging from 1:50 to 1:6400, was added to the coated plates. Bound antibodies were detected using a goat antihuman IgG alkaline phosphate-labeled antibody followed by nitrophenyl phosphate substrate. The absorbance readings were measured and quantitated against an international reference sera with known quantities of the respective antibodies to provide a specific antibody concentration in the sera tested, using a spectrophotometer capable of reading microtiter plates (spectramax 3400 PC; Molecular Devices, Sunnyvale, CA). The definition of a protective level for each of the antigens was as follows: DT, >0.10 IU/mL; TT, >0.10 IU/mL; PT, 5 enzyme-linked immunosorbent assay U [ELU]/mL; FHA, 3 ELU/mL; PRN, 5 ELU/mL; and FIM 2/3, 5 ELU/mL.

Statistical methods

The primary analysis used analysis of variance to examine whether newborns from mothers who received Tdap during pregnancy had higher concentrations of antibodies to DT, TT, PT, FHA, PRN, or FIM compared to newborns from mothers who did not receive Tdap during pregnancy. Antibody levels below the limit of detection were assigned the lower limit of detection value for that antigen. In a secondary analyses, Pearson correlation coefficients were calculated to explore the correlation between a

mother's and her newborn's antibody level.

Exploratory analysis

The correlate of immunity for the 4 pertussis antibodies is not established, but data indicate that multicomponent vaccines have increased efficacy.^{6,7} This indicates the level of individual antibodies associated with a protective effect against the individual developing the disease has not been determined, but the sum of all antibodies contribute to efficacy. Increased levels of IgG antibody to FIM, PRN, and PT have been associated with disease prevention.^{6,7} The defined protective levels were created by the detectable level of the diagnostic test.⁸ That is, if an individual's antibody was at or above the detectable level for the antibody, the individual was defined as having protection. This practice is consistently used in the literature, for tetanus and diphtheria^{9,10} and hepatitis B.^{11,12} Zaman et al¹³ studied maternal immunization with inactivated influenza vaccine and showed significant benefit to the mother and a 63% reduction in proven influenza illness in infants up to 6 months of age.

We assumed that the sum of >1 pertussis antibody would contribute to an increased protective effect.¹⁴ This assumption allowed us to pursue an exploratory analysis of the concept that increased antibody levels above a detectable level may indicate greater protection. We used multilogistic regression to test the odds that a newborn was protected from each of the potential pathogens. For the model, when an antibody level was less than the predefined protective level, the protection was coded as a 0 (representing no protection). Lastly, we used linear regression techniques to test if a newborn's antibody level could be predicted from group affiliation (Tdap or no Tdap).

RESULTS

There were no adverse reactions to the vaccine.

The current study collected serological data from 104 pregnant women and their newborns (total $n = 208$ samples). Half ($n = 52$) of the pregnant women

TABLE 2
Correlation between mothers and newborn antibody levels

Antibody	Pearson correlation coefficient (P value)
Diphtheria	0.345 (< .0001 ^a)
Tetanus	0.204 (< .001 ^a)
PT	0.158 (.055)
FHA	0.165 (.045 ^a)
PRN	0.965 (< .001 ^a)
FIM 2/3	0.293 (< .001 ^a)

FHA, filamentous hemagglutinin; FIM, fimbriae; PRN, pertactin; PT, pertussis toxin.

^a Significant at .05 level.

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TABLE 3
Newborns who had protective antibodies after mothers who did or did not receive Tdap

Outcome antibody	Mother did not receive Tdap, n (%) (n = 52)	Mother received Tdap, n (%) (n = 52)	OR (95% CI) Tdap: no Tdap	P value
Diphtheria	42 (80.8)	50 (96.2)	5.95 (1.24–28.69)	.0141 ^a
Tetanus	50 (96.2)	52 (100)	Undefined	.1533
PT	21 (40.4)	46 (88.5)	11.32 (4.10–31.24)	< .0001 ^a
FHA	49 (94.2)	50 (96.2)	1.53 (0.25–9.56)	.6467
PRN	45 (86.5)	50 (96.2)	3.89 (0.77–19.70)	.0812
FIM 2/3	44 (84.6)	51 (98.1)	9.27 (1.12–77.07)	.0146 ^a

CI, confidence interval; FHA, filamentous hemagglutinin; FIM, fimbriae; OR, odds ratio; PRN, pertactin; PT, pertussis toxin; Tdap, tetanus, reduced diphtheria, and acellular pertussis antigens vaccine.

^a Significant at .05 level.

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received the Tdap during the antepartum period, while the other half (n = 52) of pregnant women did not receive Tdap.

As seen in Table 1, newborns born from mothers who received Tdap during pregnancy had significantly higher concentrations of anti-DT (0.571 vs 1.970, $P < .001$), anti-TT (4.237 vs 9.015, $P = .004$), anti-PT (11.010 vs 28.220, $P < .0001$), anti-FHA (26.830 vs 104.15, $P = .002$), anti-PRN (24.700 vs 333.01, $P < .001$), and anti-FIM 2/3 (82.830 vs 1198.99, $P < .001$) when compared to newborns born from mothers who did not receive Tdap during pregnancy. Also, there was a significant correlation

between a mother's antibody level and their newborn's antibody level (Table 2).

Regarding protection against disease as defined in our analysis, there was a significant increase in the odds that newborns from mothers who received Tdap during pregnancy were protected against diphtheria (96.2% vs 80.8%; odds ratio [OR], 5.95; 95% confidence interval [CI], 1.24–28.69; $P = .0141$) and pertussis based on anti-PT (88.5% vs 40.4%; OR, 11.32; 95% CI, 4.10–31.24; $P < .0001$), and anti-FIM 2/3 (98.1% vs 84.6%; OR, 9.27; 95% CI, 1.12–77.07; $P = .0146$) antibody concentrations, compared to newborns from mothers who did not receive Tdap during preg-

nancy. Furthermore, the odds are >6 times higher for diphtheria, >11 times higher for PT, and 9 times higher for FIM 2/3. There was no significant difference in protection for tetanus (100% vs 96.2%; OR, undefined; $P = .1533$), FHA (96.2% vs 94.2%; OR, 1.53; 95% CI, 0.25–9.56; $P = .6467$), and PRN (96.2% vs 86.5%; OR, 3.89; 95% CI, 0.77–19.70; $P = .0812$) between the 2 groups (Table 3).

Table 4 displays the response profiles for the 2 groups. A higher percentage of newborns whose mothers received Tdap during pregnancy were in the ≥ 10 to ≤ 20 and ≥ 20 ELU/mL levels and less were in the <5 and >5–10 level for PT antibody when compared to newborns whose mothers did not receive Tdap.

Table 5 depicts the rates of protection stratified by group, if protection was defined by the next highest level of 10. The results allows an estimation of the duration of protection from maternal vaccination in the antepartum period with Tdap if it is assumed that antibody levels decay with a half-life of 30 days. When levels in Table 5 were compared to Table 3, the odds that newborns from mothers who received Tdap during pregnancy had increased protection became evident.

Table 6 suggests that group affiliation (Tdap or no Tdap) significantly predicts the level of each antibody. The level of anti-TT is 5 times higher, anti-DT is 1.4

TABLE 4
Antibody response profiles for 4 pertussis antibodies

Group	Level, ELU/mL	PT, n (%)	FHA, n (%)	PRN, n (%)	FIM 2/3, n (%)
Tdap	<5 (<3 for FHA)	6 (11.5)	2 (3.8)	2 (3.8)	1 (1.9)
	5–10 (3–10 for FHA)	12 (23.1)	1 (1.9)	3 (5.8)	3 (5.8)
	>10 and <20	10 (19.2)	6 (11.5)	3 (5.8)	1 (1.9)
	≥ 20	24 (46.2)	43 (82.7)	44 (84.6)	47 (90.4)
No Tdap	<5 (<3 for FHA)	31 (59.6)	3 (5.8)	7 (13.5)	8 (15.4)
	5–10 (3–10 for FHA)	15 (28.8)	16 (30.8)	17 (32.7)	5 (9.6)
	>10 and <20	3 (5.8)	15 (28.8)	15 (28.8)	5 (9.6)
	≥ 20	3 (5.8)	18 (34.6)	13 (25.0)	34 (65.4)
CMH χ^2 (P value)		37.3 (< .001)	27.5 (< .001)	37.4 (< .001)	10.7 (.013)

CMH, Cochran-Mantel-Haenszel; ELU, enzyme-linked immunosorbent assay U; FHA, filamentous hemagglutinin; FIM, fimbriae; PRN, pertactin; PT, pertussis toxin; Tdap, tetanus, reduced diphtheria, and acellular pertussis antigens vaccine.

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TABLE 5

Newborns who had protection to Tdap antigens using an elevated cutoff (10 instead of 5) comparing Tdap group to no Tdap group

Outcome	Mother did not receive Tdap, n (%) (n = 52)	Mother received Tdap, n (%) (n = 52)	OR (95% CI) Tda: no Tdap	P value ^a
PT	6 (11.5)	34 (65.4)	14.48 (5.2–40.4)	< .0001
FHA	33 (63.5)	49 (94.2)	9.40 (2.6–34.3)	.0001
PRN	28 (53.9)	47 (90.4)	8.06 (2.8–23.5)	< .0001
FIM 2/3	39 (75.0)	48 (92.3)	4.00 (1.2–13.3)	.0170

CI, confidence interval; FHA, filamentous hemagglutinin; FIM, fimbriae; OR, odds ratio; PRN, pertactin; PT, pertussis toxin; Tdap, tetanus, reduced diphtheria, and acellular pertussis antigens vaccine.

^a Significant at .05 level.

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times higher, anti-PT is 16 times higher, anti-FHA is 70 times higher, anti-PRN is 311 times higher, and anti-FIM is 1107 times higher for those newborns whose mother received Tdap during pregnancy.

COMMENT

This study clearly shows that pregnant women who receive Tdap during the antepartum period of pregnancy have significantly higher antibody levels in their serum at delivery to all 6 antigens in the vaccine. We also have shown that the maternal antibody is actively transferred thereby significantly elevating newborn levels. For each antigen measured there was a significant increase in the antibody

titer in the serum of mothers who received Tdap and their infants. Unfortunately, prevaccination maternal serum levels were not obtained as the study was done on discarded matched maternal and cord samples.

As a consequence of the Tdap we showed that there is a strong likelihood a significant increase in the odds that a newborn whose mother received Tdap would have protection against diphtheria and pertussis but not against tetanus. We were not surprised by the tetanus data because 50/52 (96%) of the pregnant women who did not have Tdap nevertheless had adequate antibodies present indicating past immunization.

The ACIP has preferred administration of Tdap in the postpartum period rather than intrapartum. The major focus of ACIP has been regarding the question of interference of passive maternal antibodies on neonatal active immunity.^{15,16} However, investigations by Englund et al¹⁷ did not find that maternal PT antibodies interfered with active immunization of the fetus with acellular pertussis vaccines given in the usual schedule. Additionally, in studies where high levels of maternal *Haemophilus influenzae* type B antibodies dampened infants initial response to active *Haemophilus influenzae* type B conjugate vaccine, the infant's response was no different after the primary series was complete.¹⁸

Shakib et al⁸ demonstrated that 75% of infants are born with pertussis anti-

body levels that are lower than the modest levels associated with potential protection and that 90% of infants were predicted to have little antibody by 6 weeks of life. Healy et al¹⁹ demonstrated that maternal delivery levels of IgG to PT, FHA, and FIM were extremely low and although excellent maternal transport of these pertussis antibodies occurred, the low levels in the neonates and their rapid decay left the neonate with little protection. Mooi and de Greeff²⁰ have encouraged maternal vaccination against pertussis as it offers the possibility to protect infants from birth until immunity is induced by active immunizations. Gall²¹ has advocated maternal immunization as a method of protecting the neonate from pertussis disease until active immunity is present. Edwards²² found that cord blood antipertussis IgG concentrations in an unselected population were equal to maternal levels and by 4 months of age most infants had no measurable antibody to PT or FHA. She postulated that maternal immunization would provide early protection of the newborn.

In conclusion, this paper reinforces the concept that high titers of pertussis antibodies, PT, FHA, PRN, and FIM 2/3 are transferred to the fetus when women are vaccinated in the antenatal period. If a program of maternal vaccination in the second trimester were to be adopted as a standard of practice in obstetrics there would be less risk to infants of pertussis disease in the first 5–6 months of life until their active vaccinations with Tdap at 2, 4, and 6 months of age established active immunity. ■

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TABLE 6

Antibody levels in newborns comparing mothers receiving and not receiving Tdap

Outcome	P	P value ^a
Diphtheria	1.36	< .001
Tetanus	4.59	< .001
PT	15.47	< .001
FHA	69.95	< .001
PRN	311.19	< .001
FIM 2/3	1107.13	< .001

FHA, filamentous hemagglutinin; FIM, fimbriae; P, difference in newborn antibody level; PRN, pertactin; PT, pertussis toxin; Tdap, tetanus, reduced diphtheria, and acellular pertussis antigens vaccine.

^a Significant at .05 level.

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