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Low pertussis antibody levels in maternal and umbilical cord blood samples in Turkey

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Pertussis continues to cause significant mortality and morbidity in many countries despite high vaccine coverage, especially among young infants. The aim of the study was to determine pertussis antibody levels in paired maternal and cord blood samples, to evaluate the placental transfer of these antibodies, and to assess whether newborn infants have adequate antibody levels against pertussis. Antibody titers to pertussis toxin (anti-PT) and filamentous hemagglutinin (anti-FHA) were measured by in-house enzyme linked immunosorbent assay (ELISA) in 251 paired maternal delivery and cord blood samples. Geometric mean concentrations (GMCs) of pertussis antibodies and cord:maternal GMC ratios were calculated. GMCs of maternal anti-PT and anti-FHA antibodies at delivery were 4.12 and 9.89 EU/ml, respectively. Cord GMCs were 133% and 131% of maternal delivery values for PT and FHA, respectively; demonstrating effective placental transfer. However, cord pertussis antibodies were at a low concentration; 5.49 EU/ml for PT and 12.73 EU/ml for FHA. Only 34.6% of infants had protective anti-PT levels (≥10 EU/ml) at birth. Anti-pertussis antibody concentrations were extremely low in pregnant women in Turkey where childhood pertussis vaccination coverage has been high for a long time. Despite effective placental antibody transfer, umbilical cord pertussis antibody concentrations are similarly low. A majority of young infants are vulnerable to pertussis infection until the onset of primary vaccinations. These data support the need for pertussis vaccination during pregnancy to prevent infant infection in Turkey.

Key words: cord blood, pertussis, pertussis antibodies, pregnant women, seroprevalance.

Diphtheria-tetanus-pertussis (DTP3) immunization coverage is estimated at 86% worldwide, reaching more than 90% in the 129 (66%) of 194 WHO member states[1]. However, despite this high vaccination coverage, pertussis continues to be a global public health problem. The WHO estimates that 16 million new cases of pertussis occur every year, with more than 63,000 pertussis deaths[2]. The majority of pertussis-related deaths occur in infants younger than 3 months old[3,4]. Therefore, any protection that can be provided at this age is crucial.

Protection against pertussis can best be achieved by active immunization, but the first dose of pertussis vaccine cannot be given before 6 weeks of age, because immune systems of newborn infants are not able to respond well to pertussis vaccine. Furthermore, pertussis vaccination does not offer immediate protection. It takes several days to respond to the vaccine. Even though partial protection against serious disease begins after the first dose of pertussis vaccine, full infant protection is not likely to be achieved until after the completion of primary vaccination at 6 months[5,6]. Therefore,

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in the first months of life, infants depend on maternal antibodies for protection until they develop immunity by vaccination[7].

Maternal antibodies against *B. pertussis* antigens are transferred from mother to infant across the placenta and may protect infants from severe pertussis disease for a varying period of time, depending on the level of placental transmission and the rate of decay of passively acquired antibodies[7]. There is a highly significant correlation between the level of anti-PT IgG antibody in the serum and protection against pertussis[8-10]. In the pre-vaccine era, 30-50% of pregnant women had circulating antibodies against pertussis[11]. However, in the post-vaccine era, studies carried out on women vaccinated in childhood showed low levels of pertussis antibodies in both mothers and newborns[12-16].

In Turkey, routine childhood pertussis immunization with whole cell pertussis vaccine (DTP) was started in 1968 and was administered in the 2nd, 3rd and 4th months of life, with a booster dose in the 18th month. In 2007, acellular pertussis vaccine (DTaP) was introduced, and the third dose DTaP vaccination coverage reached 97% in 2009[17]. In 2010, a childhood booster vaccine of DTaP was added to the immunization schedule for 5-6 year old children in the first grade of elementary school. However, additional boosters for adolescents or adults have not been incorporated into the Turkish immunization schedule. Cocooning strategy and pertussis vaccination during pregnancy is not yet a current practice.

Pertussis serologic data for pregnant women and newborn infants in Turkey are lacking. There is only one previously published study investigating pertussis seroprevalence in cord and maternal blood[15]. Therefore, in this study, we aimed to determine the anti-pertussis antibody levels in paired maternal delivery and cord blood samples, to evaluate the placental transfer of these antibodies, and to assess whether newborn infants born in Turkey have adequate antibody levels against pertussis.

Material and Methods

Study population

The study was carried out in 251 mother-infant pairs admitted between April and June 2011. Participants eligible for enrollment were pregnant women aged 18-45 years old, who delivered a healthy term infant ≥ 37 weeks' gestation and their term infants. Exclusion criteria for mother-infant pairs were serious underlying disease, multiple gestation or antenatal detection of a major birth defect in the infants. Infants born before 37 weeks of gestation were also excluded.

The study was approved by the Medical Ethical Committee (Meeting number 16/1 in 06.04.2011). The written informed consent was obtained from all mothers. The mothers were asked to complete a brief questionnaire collecting information on basic demographics, health, and pertussis immunization history. In addition, maternal age, gestational age, mode of delivery, gender of newborn and birth weight were recorded.

Table I. Demographic Characteristics of Mothers and Their Infants in the Study Population (n=251).

Maternal age, mean ± SD (range), years	28.2 ± 5.3 (16-45)
Age groups of mothers, n (%)	
<20 years	10 (4.0)
20-24 years	59 (23.5)
25-29 years	80 (31.9)
30-34 years	76 (30.3)
35-45 years	26 (10.4)
Mode of delivery, n (%)	
Vaginal	142 (56.5)
Cesarean	109 (43.5)
Gestational age, mean ± SD (range), weeks	39.2 ± 1.0 (37-42)
Birth weight, mean ± SD (range), gram	3370.6 ± 485.6 (2200-5000)
Sex of newborns, n (%)	
Male	124 (49.4)
Female	127 (50.6)

Table II. Geometric Mean Concentrations of Anti-Pertussis Antibodies in Maternal Delivery Serum and Cord Blood Samples and Placental Transfer Ratios.

	Geometric mean concentrations (95% CI) [range], ELISA units/ml		Placental transfer ratio (%)
	Maternal serum	Cord blood	
Anti-PT	4.12 (3.53-4.80) [0.10-151.36]	5.49 (4.58-6.53) [0.06-199.53]	133
Anti-FHA	9.89 (8.22-11.90) [0.01-208.93]	12.73 (10.66-15.79) [0.00-245.47]	131

CI: Confidence interval

Laboratory Methods

Blood samples were obtained from mothers immediately after delivery (maternal blood) and from the umbilical cord (cord blood). Paired maternal and umbilical cord blood samples were centrifuged and serum samples were stored at -80°C until analyzed. Anti-PT IgG and anti-FHA IgG antibody ELISA tests were performed at Public Health Institution of Turkey, Microbiology Reference Laboratories, Vaccine Preventable Bacterial Diseases Serology Laboratory. The standardization of this in-house ELISA for pertussis serology was studied and published previously[18]. In brief, the test was conducted by using 96-well flat-bottom plates (Greiner, 655001, Frickenhausen, Germany). Purified PT 10 μg PN/ampoule (JNIH-5, Biken, Japan) and Purified FHA 10 μg PN/ampoule (JNIH-4, Biken, Japan) were used for coating the plates. The concentration of PT and FHA antigen in 100 μl coating buffer was 0.1 μg PN/ml and 0.04 μg PN/ml, respectively. Coated plates were incubated at 4°C for 48 hours. The plates were blocked by adding 125 μl of blocking buffer (PBS containing 0.5% BSA) and incubated on an Incubator/shaker (Labsystem iEMS, Helsinki, Finland). Eight two-fold serial dilutions of test sera and reference serum (anti-Pertussis Reference human sera IgG [250 ELISA Unit (EU) for anti-PT IgG, 400 EU for anti-FHA IgG, Biken, Japan]) in PBS containing 0.5% BSA and 0.05% Tween 80 were added. Fc-specific alkaline phosphatase-conjugated goat anti-human IgG (Seikagaku, Kogyou, Tokyo, Japan) diluted in PBS-T was then added; P-Nitrophenyl phosphate (Sigma) diluted in diethanolamine buffer (1 mg/ml, pH 9.6) was then added and 3M NaOH was used as the stop solution. Plates were read at A405/630 on an ELISA reader (Labsystem, Multi Skan EX, Helsinki, Finland). The anti-PT and anti-FHA IgG antibody titers were calculated by the parallel line assay ($p = 0.05$).

Serum levels of anti-PT and anti-FHA antibodies were reported as enzyme-linked immunosorbent assay (ELISA) units per milliliter (EU/ml). The lower limit of detection for both antibodies by this method was 1.0 EU/ml. Although protective antibody levels against pertussis infection have not been clearly established, antibody levels ≥10 EU/ml were considered as a threshold for protection which is based on the lowest antibody levels among children recovering from pertussis[19]. In this study, antibody levels ≥10 EU/ml were also accepted as protected. Antibody levels ≥100 EU/ml for anti-PT antibody were considered as indicative of current or recent pertussis infection[19, 20].

Statistical Analysis

Statistical analysis was performed with SPSS version 20.0 for Windows (SPSS, Chicago, IL). Anti-PT and anti-FHA antibody concentrations in serum samples were calculated as geometric mean concentrations (GMCs) with 95% confidence intervals (95% CI). Placental transfer of pertussis antibodies was defined by calculating the ratio of antibody GMCs for umbilical cord and maternal serum. Mann-Whitney U test was used for the comparison of means between the groups. A value of $p < 0.05$ was considered as statistically significant. Multivariate regression analysis was performed to determine the influence of variables (e.g. maternal age, gestational age, birth weight and

gender of infants) on anti-pertussis antibody GMCs.

Results

A total of 251 mother-infant pairs were enrolled in the study. The mean maternal age was 28.2 years (range, 16-45 years). The mean gestational age was 39.2 weeks (range, 37-42 weeks) and the mean birth weight was 3370 g (range, 2200-5000 g). Demographic characteristics of the study population are summarized in Table I. None of the mothers received Tdap during pregnancy and none of them reported prolonged cough (lasting ≥2 weeks) or contact with a person suffering from prolonged cough during pregnancy.

Maternal and cord blood geometric mean concentrations (GMCs) for anti-PT and FHA antibodies are shown in Table II. Maternal GMCs of anti-PT and anti-FHA antibodies were 4.12 and 9.89 EU/mL, respectively. In cord blood samples, GMCs of anti-PT and anti-FHA were 5.49 EU/mL and 12.73 EU/mL, respectively. The placental transfer ratios of pertussis antibodies were 133% for anti-PT and 131% for anti-FHA. In all maternal age groups, cords had higher titers than maternal serum samples and placental transfer ratios ranged between 127% - 139% for anti-PT and 126% - 134% for anti-FHA.

When levels of ≥10 EU/ml were accepted as protective concentrations, protective levels of anti-PT and anti-FHA were found in only 25.1% and 54.6% of maternal serum samples, respectively. The percentage of protective concentrations of anti-PT and anti-FHA in umbilical cord blood samples were 34.6% and 59.0%, respectively (Table III).

Anti-PT levels ≥100 EU/ml, which is the serologic evidence of recent pertussis infection, were detected in 3 (1.2%) maternal serum samples. Infants of the 3 mothers with evidence of recent infection all had anti-PT levels of ≥100 EU/ml.

Maternal anti-PT and anti-FHA levels were significantly correlated ($p<0.001$) with umbilical cord blood anti-PT and anti-FHA levels; $r=0.798$ and $r=0.886$, respectively. There was no association between maternal anti-PT or anti-FHA levels and maternal age. There was also no association between cord anti-PT or anti-FHA levels and either gestational age or birth weight.

Discussion

In recent studies investigating pertussis seroprevalence in paired maternal and cord blood samples, maternal GMCs of anti-PT and anti-FHA were reported to range between 2.4-11 EU/ml and 6.9-26.6 EU/ml, respectively and cord GMCs for anti-PT and anti-FHA were reported to range between 4.1-21.5 EU/ml and 12.3-32.0 EU/ml, respectively[12,13,16,21-23]. In our study, the GMCs of anti-PT and anti-FHA antibodies were 4.1 and 9.9 EU/ml, respectively in maternal serum; and 5.5 EU/ml and 12.7 EU/ml, respectively in umbilical cord blood samples. Also, a recent study from Turkey observed similar low anti-pertussis antibody levels in cord and maternal blood[15]. These data suggest that even in countries where pertussis vaccine coverage for children has been high for a long time, like Turkey, anti-pertussis antibody concentrations were extremely low in pregnant women and their newborn infants.

The present study suggests that there is a strong correlation between maternal and cord anti-pertussis antibody levels. Recent studies observed higher anti-pertussis antibody levels in cord blood samples than in maternal blood samples[13,15,16,23,24]. We also observed higher anti-PT and anti-FHA antibody levels

Table III. The Percentage of Protective Levels of Anti-PT and Anti-FHA (≥10 EU/ml) in Maternal and Cord Blood Samples and the Percentage of Recent Maternal Pertussis Infection (Anti PT≥100 EU/ml) (n = 251).

	Percentage of protective antibody levels		Recent pertussis infection
Variable	Anti-PT ≥10 EU/ml	Anti-FHA ≥10 EU/ml	Anti-PT ≥100 EU/mL
Maternal serum, n(%)	63 (25.1)	137 (54.6)	3 (1.2)
Cord blood, n(%)	87 (34.6)	148 (59)	3 (1.2)

in umbilical cord blood than in maternal blood. Placental transfer to the infants in our study of pertussis antibodies was 133% for anti-PT and 131% for anti-FHA. Moreover, infants born to mothers who had serologic evidence of recent pertussis infection all had anti-PT antibody levels higher than 100 EU/ml. The efficiency of placental transfer demonstrates the potential for neonatal and infant protection against pertussis when levels of maternal pertussis-specific antibody are elevated[13]. However, despite efficient placental transfer, cord pertussis antibody levels were found to be too low. Only 34.6% of newborn infants have been protected against pertussis infection, according to the arbitrary cut-off value associated with potential protection. A majority of these infants are vulnerable to pertussis disease because levels of passively acquired maternal antibody are too low to provide any significant degree of protection.

The potential for protection of the infants exists by the provision, during pregnancy, of higher levels of pertussis antibodies[13]. Recent studies have also suggested that pertussis vaccination during pregnancy directly protects young infants through the transfer of maternal pertussis antibodies, in addition to being effective, safe, and well tolerated[25-31]. Maternal vaccination with the tetanus, diphtheria, and acellular pertussis (Tdap) during pregnancy has recently been recommended in several countries such as the United States, Argentina, Belgium, Israel, New Zealand, and the United Kingdom [4,32]. In Turkey, maternal vaccination with a tetanus toxoid (TT) was started in 1994. In 2004, the tetanus-diphtheria (Td) vaccine replaced the TT vaccine in the maternal immunization schedule and Td vaccine coverage among pregnant women reached 62% in 2012. However, pertussis vaccination during pregnancy is not a current practice in Turkey.

The present study shows that most pregnant women in Turkey have low levels of anti-pertussis antibody. Despite effective maternal antibody transfer, pertussis antibody concentrations are too low in newborn infants to provide adequate protection against pertussis infection. A majority of newborn infants are vulnerable to pertussis infection. These data support the need for pertussis vaccination during pregnancy for preventing infant infection in Turkey. It might be a practical approach to replace the Td vaccine with Tdap vaccine in the routine maternal immunization schedule of Turkey.

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