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Neonatal and maternal antibodies for pertussis
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SUMMARY

In order to clarify the pertussis immune status of the Japanese population, we investigated serum pertussis toxin (PT)-specific IgG antibody of infants and mothers between April 2016 and March 2018. A total of 206 infants (n = 22, <32 weeks of gestational age [wGA]; n = 70, 32–36 wGA; n = 114, ≥37 wGA) and 170 mothers were enrolled. Maternal seroprevalence and antibody geometric mean titer (GMT) were 52.4% and 10.7 EU/mL, respectively. Antibody GMT, seroprevalence, and mean ratio of infant to maternal antibody titers of infants at <32 wGA were 3.2 EU/mL, 13.6%, and 42.5%, respectively, and were significantly lower than those of infants at 32–36 wGA (9.7 EU/mL, 54.3%, and 110.2%) and infants at ≥37 wGA (12.1 EU/mL, 57.9%, and 112.6%). Of the 21 infants who underwent a second examination, five infants were positive in the first examination. Of those five, the GMT for PT had decreased by 52.6% on average at 4.3-week intervals. In the second examination, two infants were seropositive. Anti-PT antibody was negative in approximately half of the mothers and infants. Thus, new vaccination strategies such as the vaccination of pregnant women are needed to prevent pertussis infection in early infancy.
INTRODUCTION

Pertussis is a vaccine-preventable disease, the resurgence of which has been recognized in many developed countries (1–3). In Japan, the number of pertussis infections in adolescents and adults has increased since 2002, and large-scale outbreaks occurred in several universities in 2007 (4,5). However, adolescents and adults with pertussis are sources of infection to infants, leading to severe and life-threatening consequences (6,7).

The protection of infants against pertussis depends on the presence of maternal antibodies during pregnancy. Although the vaccination of pregnant women has recently been implemented as a measure against infantile infection in many countries (8–10), this is not a current practice in Japan. Childhood pertussis vaccination with diphtheria–tetanus whole-cell pertussis (DTwP) vaccine began in 1958 in Japan, but it was discontinued in 1975 due to serious adverse events. Acellular pertussis (aP) vaccine with fewer adverse reactions was developed in Japan (11), and DTaP vaccine was introduced in 1981. Presently, DTaP vaccination is recommended in the third, fourth, and fifth to eleventh months of life, with a booster dose at 12 to 23 months. The vaccination coverage rate has risen to over 95%, and surveys since 2010 show that most infants have been inoculated four times. However, pertussis vaccines were not hitherto administered after three years
until a booster vaccination was approved in 2018. This study seeks to determine the concentrations of antibodies against pertussis antigens in neonatal and maternal blood and to evaluate the efficacy of transplacental antibody transfer.

MATERIALS AND METHODS

Women and infants who were admitted to the neonatal intensive care unit in Konan Kosei Hospital, Aichi, Japan, between April 2016 and March 2018 were recruited. Those who gave written informed consent to participate were enrolled into the study. For twins, only the eldest infant was enrolled. Mothers who did not give consent by the time of their routine blood test were excluded from the study. Maternal serum was collected at the routine test that was usually performed on the third day after a vaginal delivery or on the seventh day after a cesarean section. Infant serum was collected at the routine test within one week after delivery. Gestational age (GA) was obtained from maternal medical records and determined by ultrasound at the early phase of the pregnancy. Serum pertussis toxin (PT)-specific IgG antibody was measured by enzyme immunoassay at SRL Limited (Tokyo, Japan) using the Pertussis Antibody Enzyme Immunoassay Kit (Denka Seiken, Co., LTD., Tokyo, Japan). The antibody levels were expressed in EU/mL and subsequently log-transformed to establish geometric mean titers (GMT). The same
measurements were performed at 4–6 weeks of age for infants hospitalized for >1 month. Anti-PT IgG level ≥10 EU/mL was defined as positive. The placental transfer ratio was defined as the ratio of infant to maternal antibody titer and was evaluated in mother–infant pairs with a maternal anti-PT IgG level of >5 EU/mL. This study was approved by the Konan Kosei Hospital Clinical Research Review Committee. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (R Foundation for Statistical Computing, Vienna, Austria) (12). The Fisher's exact test, Mann–Whitney U test, Kruskal–Wallis test, Steel–Dwass test, and Holm test were used for statistical comparisons. $P < 0.05$ was considered statistically significant.

RESULTS

A total of 206 infants and 170 mothers were enrolled in this study. Thirty-six mothers did not give consent prior to their routine blood test; therefore, only their infants were included in this study. The median maternal age was 33.9 years. Maternal seroprevalence and antibody GMT for PT were 52.4% and 10.7 EU/mL, respectively, and there were no significant differences in age ($P > 0.05$) (Table 1). There were also no significant differences in the seroprevalence and antibody GMT between primiparous and
multiparous women.

The median GA of all infants was 37.1 weeks (range 26.4–41.6 weeks), and the median birth weight was 2,373 g (range 781–4,218 g). No infants were treated with immunoglobulin therapy before the blood tests. Of the 206 infants, 22 were born at <32 weeks of gestational age (wGA), 70 at 32–36 wGA, and 114 at ≥37 wGA. Their median GA was 29.3 weeks, 35.3 weeks, and 39.0 weeks, respectively. The mean age of infants when blood was collected was 0.81 ± 0.16 weeks. Infant seroprevalence and antibody GMT for PT were 51.9% and 9.7 EU/mL, respectively. Values for infants born at <32 wGA were 13.6% and 3.2 EU/mL, respectively, and were significantly lower than those for infants born at 32–36 wGA and ≥37 wGA (P < 0.01) (Table 2).

The mean placental transfer ratio in 137 mother–infant pairs with maternal anti-PT IgG level >5 EU/mL was 104.1%. The ratio of infants born at <32 wGA was 42.5% and was significantly lower than that of infants born at 32–36 wGA and ≥37 wGA (P < 0.01) (Table 3).

The second measurement was carried out in 21 infants with a median GA of 30.0 weeks (range 27.0–39.8 weeks). Five of the twenty-one infants were seropositive in the first examination. Of those five, the mean duration between the two assays was 4.3 weeks (range 3.9–5.0 weeks), and the GMT for PT was 21.1 EU/mL in the first examination and
10.0 EU/mL in the second examination. The GMT decrease rate between the two assays was 52.6%. In the second examination, two out of the five were seropositive.

DISCUSSION

The results of this study show that the antibody against the pertussis antigen was negative in approximately half of the mother–infant dyads in a region within the northern part of the Aichi Prefecture in Japan. In addition, the antibody of infants born at <32 wGA was significantly lower than that of infants born at ≥32 wGA. There are a number of studies investigating pertussis antibodies in infants aged <6 months in Japan (13–16), but in most of these studies, the number of enrolled neonates was not determined. To our knowledge, this is the most comprehensive study evaluating neonatal antibodies against pertussis in Japan.

According to national surveys conducted every five years since 2003, which covers all age groups regardless of gender, the seroprevalence of anti-PT IgG of people in their 20's and 30's was 45.5% in 2003, 45.2% in 2008, and 70.7% in 2013 (15,16). The maternal seroprevalence of anti-PT IgG in the present study was lower than that recorded in the national survey conducted in 2013 but was close to the levels found in the previous two surveys. There were nationwide pertussis epidemics in Japan between 2007 and 2010
(4,5), which could have led to the increase in the seroprevalence observed in the national survey in 2013. Between 2008 and 2012, 57 children who had pertussis were treated at our hospital (17); however, our results were similar to those of the two national surveys conducted in 2003 and 2008. The anti-PT IgG titers and the seroprevalence of mothers in their thirties were lower than those of other age groups, although there were no statistical differences between them. DTwP vaccination was discontinued in Japan in 1975 due to the occurrence of postvaccination fatal cases. Subsequently, the nationwide pertussis epidemics occurred, and the number of pertussis-related death cases increased. A DTaP vaccination with reduced adverse reactions was introduced in 1981, and as the women in their 30's who participated in this study were born between 1982 and 1993, this was shortly after routine DTaP vaccination commenced. As such, the vaccination rate was low compared with the present period. Consequently, it is possible that the vaccine policies in Japan influenced the relatively low pertussis antibody titers observed in this study.

In the present study, the overall neonatal seroprevalence was similar to the maternal seroprevalence. Maternal antibody transfer to the fetus has been reported as starting between 13 and 17 wGA, being most active after 32 wGA (18,19). At birth, the fetomaternal ratios of PT antibodies have been reported as 0.55 before 32 wGA, 0.79 between 32 and 37 wGA, and 1.07 after 37 wGA (20). This study specifically showed
that the seroprevalence and antibody titers to PT were significantly lower in preterm infants born at <32 wGA than those born at ≥32 wGA. However, the seroprevalence and antibody titers of preterm infants born at 32–36 wGA were not significantly different compared with those of infants born at ≥37 wGA. This finding suggests that the risk of pertussis infection in relatively late preterm infants such as ≥32 wGA may be similar to that of full-term infants after 37 wGA. The birth levels of pertussis-specific IgG decline rapidly to negligible values by 2 months of age, and the half-life of maternal antibody to PT is 36 days (21,22). In the present study, many of those who were tested twice were preterm infants born at <32 wGA, and only five infants were seropositive in the first examination. Although the number of cases was small, the antibody titer to PT was halved in about 4–5 weeks, and this observation supports the findings of a previous study (21). Furthermore, many infants did not have high anti-PT IgG titers even in the seropositive cases, hence the titers becoming negative within a short period after birth. Therefore, measures such as raising the antibody titers at birth are necessary to protect infants before the vaccination from pertussis infection. Several countries introduced routine Tdap vaccination to pregnant women to increase the transfer of pertussis antibodies to infants (8–10), and this has been shown to be effective in the prevention of infantile pertussis (23,24). The recommended schedule of maternal pertussis vaccination is during the third
trimester (>26 weeks gestation), but this approach may have no effect on extremely preterm infants (8). However, recent observational studies have demonstrated that antibody transfer to both preterm and full-term infants was superior following maternal pertussis immunization during the second trimester compared with vaccination during the third trimester (25,26). Since preterm infants are most vulnerable to pertussis and this study has shown that infants born at <32 wGA are at a high risk of the infection, maternal vaccination during pregnancy earlier than the recommended period may be suitable when premature birth is expected.

There are some limitations associated with the results of this study. First, the antibody titers measured in the neonates were supposed to be lower than those at birth because the serum samples were collected nearly one week after birth. Assuming that the half-life of the infant anti-PT IgG is five weeks, the values at birth were presumed to be approximately 15% higher than the results shown in this study. Second, as this study was conducted at a regional hospital, it is possible that the results may be geographically biased.

In conclusion, across the region where this study was conducted in Japan, approximately half of the mothers and their infants at birth lacked protective antibodies against pertussis. In addition, anti-PT IgG in seropositive infants rapidly decreased after
birth. Thus, new vaccination strategies, such as the vaccination of pregnant women, are
needed to prevent pertussis infection in the early stages of infancy.

**Conflict of interest**

None to declare
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Table 1. Maternal GMT and seroprevalence of anti-PT IgG

| Age          | Total  | 20–29 years | 30–39 years | 40–49 years |
|--------------|--------|-------------|-------------|-------------|
| N            | 170    | 46          | 107         | 17          |
| GMT (95% CI), EU/mL |       |             |             |             |
|              | 10.7   | 12.4        | 9.7         | 14.2        |
| (7.4–14.1)   | (7.9–16.9) | (4.7–14.4) | (4.2–24.2) |             |
| Seroprevalence, % | 52.4  | 60.9        | 46.7        | 64.7        |

Ratio by antibody values, %

| 0–9 (EU/mL) | 47.6 | 39.1 | 53.3 | 35.3 |
| 10–19       | 21.8 | 26.1 | 19.6 | 23.5 |
| 20–29       | 10.6 | 17.4 | 8.5  | 5.9  |
| 30–49       | 14.1 | 15.2 | 12.1 | 23.5 |
| 50–99       | 4.1  | 2.2  | 3.7  | 11.8 |
| ≥100        | 1.8  | 0    | 2.8  | 0    |

GMT, geometric mean titer; PT, pertussis toxin; CI, confidence interval
Table 2. Neonatal GMT and seroprevalence of anti-PT IgG

| Gestational age | Total | <32 weeks | 32–36 weeks | ≥37 weeks |
|----------------|-------|-----------|-------------|-----------|
|                | 206   | 22        | 70          | 114       |
| GMT (95% CI), EU/mL |       |           |             |           |
|                | 9.7   | 3.2 *     | 9.7         | 12.1      |
| (7.0–12.5)     | (-3.0–9.4) | 6.3–13.0 | (7.8–16.4)  |
| Seroprevalence, % | 51.9  | 13.6      | 54.3        | 57.9      |
| Ratio by antibody values, % |       |           |             |           |
| 0–9 (EU/mL)    | 48.1  | 86.4      | 45.7        | 42.1      |
| 10–19          | 25.2  | 4.5       | 31.4        | 25.4      |
| 20–29          | 9.7   | 0         | 8.6         | 12.3      |
| 30–49          | 11.2  | 4.5       | 11.4        | 12.3      |
| 50–99          | 4.9   | 4.5       | 2.9         | 6.1       |
| ≥100           | 1.0   | 0         | 0           | 1.8       |

GMT, geometric mean titer; PT, pertussis toxin; CI, confidence interval
*p < 0.01; <32 weeks vs. 32–36 weeks; and <32 weeks vs. ≥37 weeks

Analysis used was the Kruskal–Wallis test, Steel–Dwass test, and Holm test.
Table 3. Placental transfer ratio of anti-PT IgG

| Gestational age | Total  | <32 weeks | 32–36 weeks | ≥37 weeks |
|-----------------|--------|-----------|-------------|-----------|
| N               | 137    | 15        | 47          | 75        |
| Placental transfer ratio, % | 104.1 | 42.5* | 110.2 | 112.6 |

PT, pertussis toxin

*p < 0.01; <32 weeks vs. 32–36 weeks; and <32 weeks vs. ≥37 weeks

Analysis used was the Kruskal–Wallis test, the Steel–Dwass test, and the Holm test.