SHORT COMMUNICATION

Seroprevalence of *Bordetella pertussis* Antibodies to Pertussis Toxin Among Healthy Children

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ABSTRACT

Pertussis or whooping cough is a highly communicable, vaccine-preventable acute respiratory tract disease, caused mainly by *Bordetella pertussis*. In this study, serosurveillance of IgG antibodies against pertussis toxin was carried out in healthy children, aged 3 months to 12 years, from the multiethnic cities of Islamabad and Rawalpindi, Pakistan. Kruskal-Wallis test was applied to determine the difference among groups. The results showed that the average values of pertussis toxin IgG dropped down significantly with age after getting the first dose in first 2 years ($p < 0.001$). Therefore, the researchers suggest introduction of booster vaccination with DTaP at second year of life and school going age to reduce the risk of getting *B. pertussis* infection.

Key words: *Serosurveillance. Bordetella pertussis. Pertussis toxin. IgG antibodies. Children. Vaccination.*

Pertussis or whooping cough is an acute respiratory tract disease caused by gram negative bacterial species *Bordetella pertussis* and *Bordetella parapertussis*. *B. pertussis* accounts for majority of pertussis cases worldwide and the DTP vaccine is primarily aimed at protecting against this bacterial species.[1] Furthermore, infections caused by *B. parapertussis* are milder as compared to *B. pertussis* infections. The incidence of pertussis has been reduced to a larger extent after mass vaccination with DTP vaccine in both developed as well as developing countries, nonetheless *B. pertussis* circulation continues to be reported in both vaccinated and unvaccinated areas. The incidence of pertussis is higher in unvaccinated populations.[1,2] Resurgence of pertussis infections has been reported from a number of countries like Netherlands, Finland, USA and Canada.[1,3,4] There are also increasing reports of pertussis cases in the adolescents and adults. Moreover, atypical infections are also of great concern, which remain largely unrecognized and undiagnosed by physicians. Adult as well as atypical and unrecognized cases act as a reservoir for close contacts, especially infants and children.[1,3]

Natural infection with *B. pertussis* develops long-lived acquired immunity to subsequent infections. Both humoral as well as cellular arms of immunity play their role in conferring protection against *B. pertussis* infection. Antibodies produced against a number of bacterial antigens such as pertactin, pertussis toxin, fimbriae, lipopolysaccharide, filamentous haemagglutinin etc., play a protective role against *B. pertussis* infection by blocking bacterial attachment in the mucosal tract, neutralizing their toxins and enabling bacterial uptake by macrophages. However, there is no consensus over the type of antigen against which the antibody is raised and its protective levels.[1]

Vaccine induced immunity wanes after 4 – 6 years and children remain susceptible to *B. pertussis* infections. Serosurveillance with ELISA for the detection of antibodies raised against *B. pertussis* is a widely used strategy for the monitoring of vaccine efficacy as well as infection with *B. pertussis*. Antibodies raised against pertussis toxin (PT) of *B. pertussis* are an indication of both the protection against the organism as well as infection. As pertussis toxin (PT) is only produced by *B. pertussis* species, ELISA for the detection of antibodies raised against it rules out the possibility of cross reactivity with *B. parapertussis* antibodies.[1,4]

The aim of the present study was to carry out serosurveillance of *B. pertussis* infections by measuring PT IgG level in the sera collected from children of age group 3 months to 12 years. A comparison between the median titer values of PT IgG at different age groups was made to study the vaccine efficacy and the ages of waning immunity (Table I).

A total of 220 blood samples were collected from children aged 3 months to 12 years (average age = 5.45 ± 4.12 years) attending the OPD of Pakistan Institute of Medical Sciences in Islamabad city during the period of August to September 2010. Most of the participants were the multiethnic population of Islamabad and Rawalpindi districts. The participants were divided into seven age groups; (1) = prevaccination; (2) = ≥ 2 years; (3) = 2 – 4 years; (4) = 4 – 6 years; (5) = 6 – 8 years; (6) = 8 – 10 years and (7) = 10 – 12 years. Information

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Table I: Median values of pertussis toxin IgG [IU/ml] in children of different age groups (3 months to 12 years). There is a rise in the median value of the IgG in the recently vaccinated group (groups 2, vaccinated ≥ 2 years), which drops in the subsequent groups due to the waning of immunity.

Groups	Size (n)	Age (months)	Vaccination	IgG (IU/ml) median	Fold change[1]
1	31	01-24	No	21.0	1.00
2	31	01-24	Yes	29.0	1.38
3	25	25-48	Yes	12.5	0.60
4	26	49-72	Yes	14.0	0.67
5	22	73-84	Yes	18.5	0.88
6	35	85-120	Yes	12.0	0.57
7	33	121-144	Yes	12.0	0.57

[1]Fold change was calculated with respect to group 1 (pre-vaccinated).

regarding age, gender, weight, vaccination status, cough history of each child and any other family member with persistent or non-persistent cough was recorded at the time of sample collection. All children in groups 2-7 had received three doses of DTP vaccine. It is to be noted that no booster dose is recommended by EPI in Pakistan. Informed verbal consent was taken from the parent or guardian at the time of sample collection. The blood samples were transported to the diagnostic laboratory of the same hospital where serum was extracted. Enzyme linked immunosorbent assay (ELISA) was performed using a commercially available kit (Virion-Serion GmbH, Germany) for pertussis toxin IgG (PT IgG) detection using manufacturer's protocol. The lower and upper cutoffs of the kits were 20 and 30 IU/ml respectively.

The data was not normally distributed so median values were used as a representative of central tendency in all groups (Table I). A non-parametric, Kruskal-Wallis test was applied to test if the median of all the groups were identical. The median of all the groups differed significantly ($p < 0.0001$). The median values of PT IgG level in each age group showed the highest value of titer in the vaccinated group of age 2 years or less (29 IU/ml). This represents a 1.39 fold increase in antibody titer in the recently vaccinated age group from pre-vaccinated individuals (Table I). However, the values of IgG titer dropped down in all groups except group 5 from group 2 (Table I). The seropositivity to pertussis toxin ELISA kit using the cutoffs of the ELISA kit was 27.6%, 51.35%, 25.9%, 18.5%, 39.4%, 14.8%, and 22.6% for each group from 1 through 7 respectively.

DTP vaccination was introduced in Pakistan in 1979 and there has been an increase in its coverage since its introduction. DTP vaccine is administered at 6, 10 and 14 weeks and there is no booster dose recommended by EPI in Pakistan. Although there has been a marked reduction in the reported *pertussis* cases since the introduction of vaccination, it still causes significant number of infections in both vaccinated and non-vaccinated children.[5] It has previously been reported that the vaccine-induced immunity wanes in children over a period of time making them vulnerable to *B. pertussis* infections.[1,3] Infants acquire IgG from mothers through transplacental passage which diminishes after some time.[1] The IgG level in group 2 indicates maternal IgG which was supposed to decline in the age group 2. However, group 2 shows highest level of PT IgG titer due to recent vaccination. However, absolute cut-off values for recent infection and the vaccine induced immunity have not so far been established. In this study, a significant drop in the titer values from group 2 to groups 3, 4 and 6 indicates waning humoral immunity and, therefore, vulnerability of the children to *B. pertussis* infections.

The results of this study are in agreement with the previous reports. Booster dose of DTP vaccine is administered in many countries of world including USA, Canada and European countries.[1] Therefore, we recommend the introduction of booster vaccination in the second year of life as well as at school going age or before to protect children at these ages.

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