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Original Article

Evaluation of anti-diphtheria toxoid antibody persistence in school-aged children in Jakarta, Indonesia

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Abstract

Background Diphtheria can be effectively prevented by adequate immunization. A combined vaccine against diphtheria toxoid, pertussis, and tetanus toxoid (DPT) is currently used in routine pediatric immunizations. Outbreaks of diphtheria could emerge in Indonesia as a consequence of declining routine vaccination during the COVID-19 pandemic.

Objective To analyze the impact of the first (administered at 18-24 months of age) and second diphtheria boosters (administered at 5-7 years of age) in retaining protective levels of anti-diphtheria toxoid antibodies. We also investigated for relevant factors associated with anti-diphtheria toxoid antibody titers.

Methods This cross-sectional study was conducted in the Senen District of Jakarta, Indonesia. The inclusion criteria were healthy children aged 6 to 7 years with documented history of DPT vaccination. Primary vaccination defined as 3 doses of DPT at age less than 1 year , first booster was DPT vaccination at 18-24 years of age, and second booster was diphtheria-tetanus (DT) vaccination received at 5 to 7 years of age. Peripheral blood specimens were obtained from participating children, after informed consent was provided by their parents. Antibodies against diphtheria in sera specimens were assessed by commercial anti-diphtheria toxoid immunoglobulin G (IgG) enzyme-linked immunosorbent assay.

Results There were 154 children included in the study, with a female majority (61%). Overall, specific humoral immunity against diphtheria was observed in 113 children (73.4%). There was no statistical difference in immunity level between genders. Importantly, children who received the first and second diphtheria booster had significantly higher anti-diphtheria antibody level than those who did not receive both diphtheria booster ($P<0.001$).

Conclusion Booster vaccinations are crucial among school-age children in Indonesia to improve their anti-diphtheria immunity and to minimize a risk of diphtheria outbreaks.**[Paediatr Indones. 2024;64:447-53; DOI: https://doi.org/10.14238/pi64.5.2024.447-53].**

Keywords: *diphtheria; anti-diphtheria toxoid; children; booster vaccination*

Diphtheria is an infectious disease with high mortality caused by exotoxin-producing Corynebacterium diphtheriae. Direct contact, sneezing, or coughing are the modes of transmission among humans. *C. diphtheriae* tends to colonize the upper respiratory tract, where it can ulcerate the mucosa and form inflammatory pseudomembranes. Of note, diphtheria toxin is the main virulence factor of *C. diphtheriae*. Upon absorption, diphtheria toxin inhibits ribosomal protein synthesis and causes cell death, resulting in damage of various organs and death.[1,2]

Diphtheria can be effectively prevented by adequate herd immunization. A combined vaccine against diphtheria toxoid, pertussis, and tetanus toxoid (DPT) is currently used in routine pediatric vaccinations. The vaccine is administered intramuscularly in three doses within the first 6 months

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of life.[1] It has been postulated that vaccine coverage must be 80-85% or higher in certain populations to provide herd immunity against diphtheria.[3] The *Expanded Programme on Immunization* (EPI) by the *World Health Organization* (WHO), therefore, recommended three doses of DPT vaccine, as a routine series of primary DPT vaccinations, followed by the first DPT booster at 18-24 months of age, and a second diphtheria-tetanus booster at 5 to 7 years.[3]

Recommendations for diphtheria vaccination by the Indonesian government were implemented during different policy periods. The Indonesian National Immunization schedule included primary vaccination for babies under 1 year old in 2009, while the policy for the first booster in children aged 18-24 months using diphtheria-whole cell pertussis-hepatitis B-*Haemophilus influenzae type B* (DPwT-HB-HIB) was implemented in 2014. In 2017, there was a diphtheria outbreak with the highest distribution in children aged 5-9 years at 32.5%. As a response, from 2017 and onwards, the Ministry of Health recommended a second booster immunization for school-age children using the diphtheria-tetanus (DT) vaccine, with an addition of tetanus diphteria (Td) booster for children aged 7 years and above.[4,5] Therefore, the longer-term persistence of immunity status for diphtheria in school-age children is questionable.

Diphtheria remains a major health problem in countries with poor vaccination coverage.[3] Diphtheria predominantly affects non-immunized pediatric populations under 15 years of age.[1] During 2011-2015, Southeast Asian countries, including Indonesia, contributed 55-99% to the total reported cases.[6] In 2018, the WHO recorded a diphtheria outbreak of 16,611 cases, of whom 1,026 cases were reported in Indonesia.[7] The insufficiency of immunization coverage among Indonesian children was partially attributed to inadequate health services across the archipelago.[8]

The COVID-19 pandemic further reduced routine pediatric immunization coverage worldwide. In 2021, routine DPT vaccination decreased to 81% in Indonesia, its lowest level since 2008.[9] As a result, diphtheria outbreaks occurred in some areas of Indonesia in 2023, including the province of West Java where an outbreak was reported, followed by 7 diphtheria-related deaths.[10]

Reports on immunity level against diphtheria among Indonesian children are still lacking. A previous study reported a high level of protection against diphtheria among Indonesian children one month after the first DT booster at 18-24 months of age.[11] However, the question remained as to whether the DT booster could sustain the level of protection against diphtheria in children at the age of 5-7 years. Here, we report our assessment of anti-diphtheria antibody levels among healthy children living in Jakarta, Indonesia who received complete routine DPT vaccinations. We specifically analyzed the impact of the first immunization (administered at 18-24 months of age) and second DT booster (administered at 5 years of age) in retaining protective levels of anti-diphtheria toxoid antibodies.

Methods

This cross-sectional study was conducted in August 2023 in Jakarta, Indonesia. Cluster random sampling was applied and 8 district wards were randomly assigned. The study was conducted in 13 pre- and elementary schools. Parents/guardians were informed about the study and those who were willing to allow their children to participate provided written informed consent. Subjects' parents filled sociodemographic data questionnaires that included parental occupation, income, and education, as well as the DPT vaccination status of their children. The inclusion criteria were healthy children aged 6-7 years with documented history of DPT vaccination. The exclusion criteria were children with known immune system disorders or malignancies, as well as children who had received immunosuppressive therapy or blood products within the 3 months prior to the study.

Primary vaccination was defined as 3 doses of DPT at age less than 1-year-old. While the first booster was DPT vaccination at 18-24 years of age, the second booster is diphtheria-tetanus (DT) vaccination received at 5 to 7 years. Complete routine DPT vaccination was defined if the subject received 5 shot of diptheria vaccination, such as 3 doses of primary vaccination, 1 dose of first booster and 1 dose of second booster. Catch up vaccination defined as a booster vaccination given at 5-year-old and above, when the child did not receive the first booster at 18-24 months of age.

Blood specimens were collected by experienced phlebotomists. Blood sera were harvested and refrigerated at -80°C prior to testing for the presence of diphtheria toxoid immunoglobulin G using a commercial anti-diphtheria toxoid enzyme-linked immunosorbent assay (*Euroimmune,* Lubeck, Germany).

The levels of anti-diphtheria toxoid antibodies were stratified into four levels based on the WHO standard: < 0.01 IU/mL (no protection), 0.01-0.09 IU/mL (uncertain protection), 0.10-1.00 IU/mL (full protection), and >1 IU/mL (long term protection).3 While "no protection" and "uncertain protection" were grouped into "susceptible" (<0.10 IU/mL), the "full protection" and "long-term protection" were grouped into "relatively immune" (≥0.10 IU/mL).

The minimum required sample size was calculated to estimate the proportion of the fully protected group, based on the results of a previous study[12] with the proportion of 64%, 0.1 precision, 5% level of significance, and 80% power, as well as the formula of a single proportion. A minimum of 88 subjects were required for this study.

Statistical analyses were performed using *IBM SPSS Statistics for Windows version 26.0* (*IBM Corp.*, Armonk, NY, USA) or *GraphPad Prism version 10.1.0* (California, USA). Descriptive data were presented as frequency and percentage for categorical variables. Since data distribution was not homogenous, continuous variables were presented as median and range. A comparison among two or more variables was analyzed by Kruskal-Wallis test. A significant result ($P<0.05$) was followed by Mann-Whitney test. The study protocol was approved by the Health Research Ethics Committee, Faculty of Medicine at Universitas Indonesia.

Results

There were 154 school-aged children who completed the primary DPT vaccination series. Among these, 16 children received only the primary DPT vaccination, 14 children received the primary DPT and catch-up booster, 61 children received the primary DPT and first booster, and 63 children received the primary DPT, first, and second boosters (**Figure 1**). The majority of participants were female (61%), and had low-middle income socioeconomic status (84.4% of parents had income less than minimum wage in

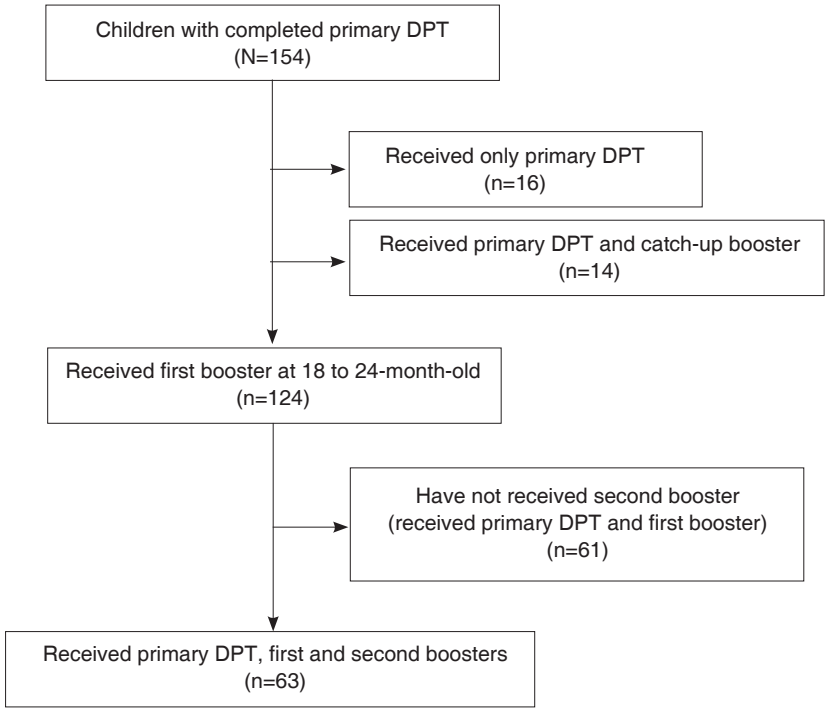

Figure 1. The description of the study participants

Jakarta, Indonesia) (**Table 1**).

According to WHO standard for immunity status of diphtheria, 73.4% subjects were relatively immune to diphtheria. If classified into four groups according to anti-diphtheria immunoglobulin G, 49.4% subjects have already had full protection of diphtheria, as shown in **Table 2**.

We observed that while the lowest median anti-diphtheria toxoid IgG was in the primary DPT group (median 0.055 IU/mL), the highest median anti-diphtheria toxoid IgG was in the primary DPT, first, and second booster group (median 0.935 IU/mL). Upon comparison among all groups, the differences in titers of anti-diphtheria toxoid IgG were significantly different (P<0.001) (**Table 3**). Subsequent analyses among the 4 groups demonstrated that the anti-diphtheria toxoid IgG concentrations in the primary DPT-only group were significantly lower than the ones in other groups who received booster vaccinations (**Figure 2**). In addition, anti-diphtheria toxoid IgG titer in the complete primary DPT + first + second booster group was significantly higher than the titer in the primary DPT + first booster group (P<0.001). However, the anti-diphtheria toxoid IgG titer in the complete primary DPT + first + second booster group was not significantly different than the titer in the primary DPT including catch-up booster group (P=0.258) (**Table 4**). This discrepancy might be partly explained by the timing of anti-diphtheria toxoid IgG measurements, which was close to the timing of second or catch-up boosters.

Sera from 154 children were divided into 4 groups received only primary DPT (n=16), the group received primary DPT and first booster (n=14), the group received primary DPT, first and second boosters (n=63) as well as the group received primary DPT and catch-up booster (n=61), were assessed for anti-diphtheria toxoid IgG antibodies (**Figure 2**).

Table 1. Demographics of study participants

Variables	(N=154)
Median age (range), months	83 (72-94)
Gender, n(%)	
Male	60 (39.0)
Female	94 (61.0)
Parental employment status, n(%)	
Employed	71 (46.1)
Unemployed	83 (53.9)
Parental income,* n(%)	
Equal or above the minimum wage	24 (15.6)
Below the minimum wage	130 (84.4)
Children's diphtheria vaccination status, n(%)	
Received primary DPT only	16 (10.4)
Received primary DPT + first booster	61 (39.6)
Received primary DPT + first + second boosters	63 (40.9)
Received primary DPT + catch-up booster	14 (9.1)

*Monthly minimum wage in Jakarta for January 2023 was IDR 4,901,798

Discussion

COVID-19 had a substantial impact on worldwide public health services by interfering in the routine provision of life-saving vaccines.[13] As a result, millions of children were at high risk of contracting vaccine-preventable diseases, such as diphtheria. According to WHO and UNICEF findings collected during

Table 2. Immune status of participants according to their anti-diphtheria antibodies

Variables	(N=154)
Status	
Susceptible, n(%)	41 (26.6)
Relatively immune, n(%)	113 (73.4)
Anti-diphtheria immunoglobulin G, n(%)	
No protection (<0.01 IU/mL)	8 (5.2)
Uncertain protection (0.01–0.09 IU/mL)	33 (21.4)
Full protection (0.10–1.00 IU/mL)	76 (49.4)
Long-term protection (>1.00 IU/mL)	37 (24.0)

Table 3. Association between diphtheria booster status and anti-diphtheria toxoid antibody titers

Variables	Median anti-diphtheria IgG (range), IU/mL	P value*
Received only primary DPT	0.055 (0.001-1.000)	< 0.001
Received primary DPT + first booster	0.363 (0.019-1.886)	
Received primary DPT + first + second boosters	0.935 (0.001-2.000)	
Received primary DPT + catch-up booster	0.629 (0.001-2.000)	

*Kruskal Wallis test was performed. IgG, immunoglobulin G

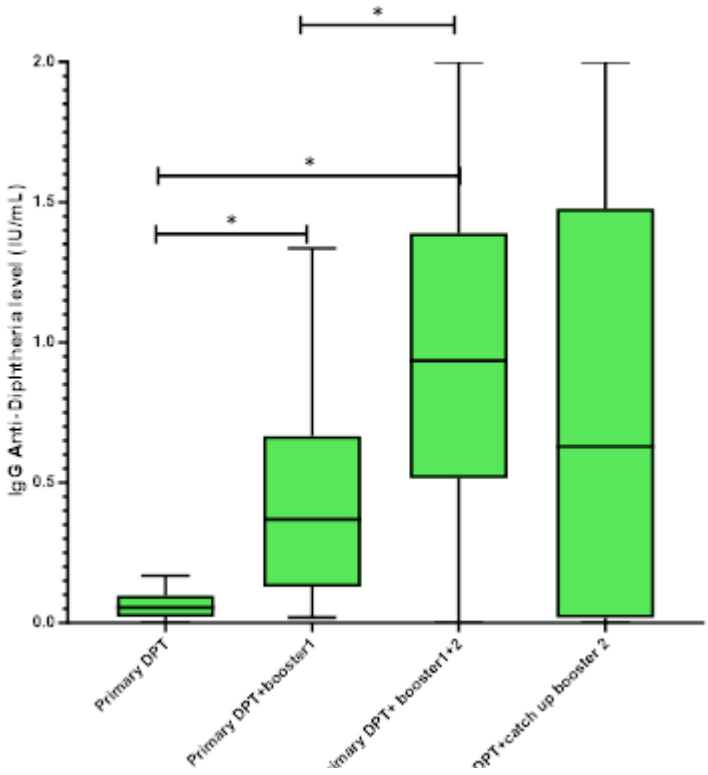

Figure 2. Anti-diphtheria toxoid immunoglobulin G titers in participants.
Solid horizontal lines inside the boxes refers to the median value. Mann-Whitney tests were performed to determine any statistically significant differences between two groups; *= P< 0.05.

Table 4. Mann-Whitney post-hoc analysis

Variables	P value
Received only primary DPT vs. received primary DPT + first booster (P<0.001)	<0.001
Received only primary DPT vs. received primary DPT + first + second boosters	< 0.001
Received only primary DPT vs. received primary DPT + catch-up booster	0.104
Received primary DPT + first booster vs. received primary DPT + first + second boosters	<0.001
Received primary DPT + first booster vs. received primary DPT + catch-up booster	0.796
Received primary DPT + first + second boosters vs. received primary DPT + catch-up booster	0.258

the COVID-19 pandemic, more than half of 129 countries (53%) reported severe disruptions of routine immunizations in the pediatric population, including the DPT primary and booster vaccinations.[14]

History has shown that diphtheria outbreaks usually occur following decreased coverage of DPT vaccinations. For example, the 2017 diphtheria outbreak in Indonesia occurred after decreased DPT vaccination coverage.[7] In 2023, several diphtheria outbreaks with 7 fatalities were reported in Indonesia, forcing the Indonesian government to ramp up its efforts to tackle the decline in childhood immunizations.[10,15]

Serological surveys are commonly performed to determine the duration of immunity after primary and booster vaccinations. Such surveys can provide important information to develop and implement public health initiatives to minimize immunity gaps.[16] However, serological surveys are seldom conducted in low- and middle-income countries due to resource constraints and limited access to relevant clinical laboratories. The serological assessment for diphtheria

is routinely performed by measuring anti-diphtheria toxoid immunoglobulin G antibody.[3,12]

In our study, 113 children (73.4%) were deemed to have immune protection from diphtheria. This finding was contrast if compared to Malaysia, that only 42.5% of children aged 5-6 years had protection against diphtheria;17 while in India, only 29.7% of children aged 5-17 years were immune to diphtheria.[18]

Of note, our study was conducted after the Indonesian Ministry of Health's routine vaccination program for school-age children (*Bulan Imunisasi Anak Sekolah*) in 2017.[19] This program was recommended to give 1 dose of diphtheria-tetanus vaccine irrespective to the child immunization status in 5 to 7 years of age. The association between children's diphtheria booster status and anti-diphtheria toxoid IgG titers was significant, with the highest median IgG titers observed in the complete primary DPT + first + second booster group. As expected, this group had significantly higher titers than those who received only the primary DPT [0.935 *vs.* 0.055 IU/mL, respectively (P<0.001)]. Interestingly, children who missed the first booster but received the catch-up booster at 5-7 years of age had antibody titers that were not significantly different from titers in those who completed two booster vaccinations [0.629 *vs.* 0.935 IU/mL, respectively (P=0.258)]. This finding underscores the importance of diphtheria boosters in school-age children to prevent the risk of an outbreak in Indonesia. This finding might be partly explained by the timing of anti-diphtheria toxoid IgG measurements, which were close to the administration of the second or catch-up booster. However, a follow-up study will be required to assess whether the higher titers will endure for a long time. This finding underscores the importance of diphtheria catch-up boosters at the pre-school age to achieve sustainable protection against diphtheria among children who missed their first booster.

In conclusion, our study provides supporting data that highlight the importance of first and second DPT boosters to maintain immunity against diphtheria among pre-school and school-age children. Diphtheria catch-up vaccines given to pre-school age children who had missed their first booster can significantly elevate their anti-diphtheria toxoid immunoglobulin G titers.

Conflict of interest

None declared.

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