PROFILE OF ANTI-DIPHTHERIA TOXOID IMMUNOGLOBULIN G AMONG PRE-SCHOOL CHILDREN IN THE SENEN DISTRICT, JAKARTA, INDONESIA

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Abstract

Background: Diphtheria is a re-emerging disease in Indonesia that can be prevented by vaccination. This study evaluates the anti-diphtheria toxoid immunoglobulin G levels among healthy children aged 6 – 7 years old who had not received the second booster immunization for Diphtheria, Tetanus, and Pertussis (DTP) commonly administered at 5 – 7 years old. **Methods:** This cross-sectional study was conducted in the Senen district of Jakarta, Indonesia. All subjects had been vaccinated with three doses of immunization for DTP during the first year of life, and subsequently classified into a group of children who received the first booster immunization of DTP at 18 – 24 months old and who had not received it yet. Antibody against Diphtheria toxoids within the sera samples was assessed by commercial Anti-Diphtheria toxoid IgG Enzyme-Linked Immunosorbent Assay and classified in four groups: no protection (< 0.01 IU/ml), uncertain (0.01 – 0.09 IU/ml), full protection (0.10 – 1 IU/ml) and long-term protection (>1 IU/ml). **Results:** Eighty-nine children were included in this study; only 71 subjects (79.7%) had received the first DTP booster at 18–24 months old. The specific humoral immunity against diphtheria was observed among 57 children (64%). Notably, among the 89 subjects, only two subjects not receiving the first DTP booster still had antibody protection for diphtheria (p=0.001, OR 27.5, 95% CI: 5.71 – 132.42). **Conclusion:** The first diphtheria booster vaccination at 18 – 24 months old is crucial to protect pre-school children against diphtheria.

Keywords

Diphtheria, anti-IgG toxoid, pre-school children, booster vaccination

Introduction

Diphtheria is one of the most feared infectious diseases globally, causing an epidemic among pediatric populations in many countries. After the establishment of three doses of the Diphtheria-Tetanus-Pertussis (DTP3) vaccine in the Expanded Programme on Immunization (EPI) by the World Health Organization (WHO), the incidence of diphtheria has decreased dramatically worldwide.[1] However, in Indonesia, diphtheria outbreaks still occurred, such as the nationwide diphtheria outbreak in 2017 that caused 596 cases with 30 deaths from 21 provinces.[2] One of the possible reasons for this outbreak was the low coverage of diphtheria vaccination and its unequal distribution in Indonesia. The coverage of DTP3 vaccination only reached 85% in 2013, and the vaccine distribution was not equal in every village and district.[3]

Due to the COVID-19 pandemic, the coverage of routine childhood immunization was unfortunately decreased. In 2021, the DTP3 vaccination fell to 81%, reaching its lowest level since 2008.[4] The problem of decreasing immunization coverage among Indonesian children was worsened by poverty, overcrowded environment, and inadequate health services.[5] As a result, the diphtheria outbreak occurred in some areas of Indonesia in 2023, e.g., an outbreak reported in West Java after seven deaths.[6]

The Ministry of Health of the Republic of Indonesia, following the recommendation of WHO, recommends DTP3 at the age of 6, 10, and 14 weeks, followed by a booster in the second year of life.[1] Study following the diphtheria booster in 18 – 24 months of age showed a high level of protection one month after the booster,[7] but the waning of the antibody has not been determined yet in the age of pre-school years.

Indeed, the immunity level against diphtheria among Indonesian children is not commonly evaluated, which could increase the risk of diphtheria outbreak in Indonesia. This study evaluates the anti-diphtheria toxoid immunoglobulin G levels among healthy children aged 6 – 7 years old who had not received the second booster immunization for Diphtheria, Tetanus, and Pertussis (DTP) commonly administered at 5 – 7 years old. We analyzed various factors associated with the antibody levels, such as gender and the first booster of DTP vaccination administered at 18 – 24 months old.

Materials and Methods

Study Design and Sampling

This cross-sectional study was a part of the study for SARS-CoV-2 surveillance in children that was conducted in August 2023 in Senen District, Jakarta, Indonesia.

We used serum samples of children who have yet to receive the second booster for DTP based on the questionnaires and the immunization diary of the children. There were 13 pre-schools and elementary schools involved in this study. Parents of the eligible children were informed about this study and contacted by teachers to fill out the offline questionnaires. The questionnaires included sociodemographic data such as parental occupation, income, education, and children's DTP vaccination status. Those who were willing to participate gave their written consent.

Healthy children aged 6 – 7 years old with a documented history of 3 doses of DTP vaccine in the first year of life (DTP3), with and without the first DTP booster at 18 – 24 months old, were included in the study. Exclusion criteria in this study were children with known immune system disorders, malignancy, receiving immunosuppressive therapy or blood product within the previous three months of the study.

An experienced phlebotomist collected blood samples, and the sera samples were refrigerated at -80^{o}C until the antibody test was performed. The serum was tested for IgG antibodies of diphtheria toxoid using commercial anti-diphtheria toxoid enzyme-linked immunosorbent assay (ELISA) (Euroimmune, Germany). The anti-diphtheria antibodies level was evaluated based on the WHO standard, which divides it into four levels: < 0.01 IU/ml (no protection), 0.01 – 0.09 IU/ml (uncertain protection), 0.10 – 1 IU/ml (full protection), and >1 IU/ml (long term immunization protection) (WHO immunity basis, 2009). Further analysis was also performed by dividing the antibody level into two groups only: immune protection present (≥ 0.10 IU/ml) and susceptible (< 0.10 IU/ml).

Ethics Approval

The study protocol was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia, with protocol number 22-09-1080 (KET-1160/UN2.F1/ETIK/PPM.00.02/2022).

Sample Size

The sample size was calculated to estimate the proportion of the fully protected group. Based on the result of the previous study[8] with a proportion of 64% and precision of 0.1, and with a 5% level of significance and 80% power with the formula of single proportion, we calculated that a minimum of 88 subjects was required.

Statistical Analysis

The statistical analysis was performed using the IBM SPSS Statistics for Windows ver. 26.0 (IBM Corp., Armonk, NY, USA). Descriptive data were presented

as frequency and percentage for the categorical variables. The data distribution was not homogenous, so the continuous variables were presented as median and minimum to maximum values. A comparison between two numerical-independent variables was tested using the Mann-Whitney test. A comparison of two categorical variables was tested using the Chi-Square test. For these tests, a p-value below 0.05 was considered statistically significant.

Results

Eighty-nine children participated in the study, with the majority being females (57; 64%). The socioeconomic status of the subject population was low-middle, with 84.3% of the parents having income less than the minimum wage in Jakarta, Indonesia. Most participants had received the first DTP-booster, as 71 children (79.8%) were vaccinated at 18 – 24 months old (Table 1).

Table 1. Demographics of The Recruited Subjects (n=89).

Variable	n (%)
Gender	
Male	32 (35.9)
Female	57 (64.1)
Parent Employment status	
Employed	49 (55.1)
Unemployed	40 (44.9)
*Parent Income**	
Below the minimum wage	71 (79.8)
Equal or above the minimum wage	18 (20.2)
Parent Education Level	
Elementary - junior	18 (20.2)
High school	50(56.2)
University of bachelor degree	21(23.6)
Children's DTP booster vaccination status	
Received booster DTP at 18 – 24 months old	71 (79.8)
Not received booster DTP at 18 – 24 months old	18 (20.2)

*Minimum wage in Jakarta for January 2023 is IDR 4,901,798

Overall, the specific humoral immunity against diphtheria was observed among 89 children, with seven children (7.9%) having long-term protection, 50 children (56.1%) having full protection, 28 (31.5%) having uncertain protection and 4 (4.5%) had no protection. (Table 2)

Table 2. Diphtheria Immunity in Study Population

Sample	Long term immunisation protection	Immunisation protection	Uncertain protection	No protection
Male (n= 32)	5 (15.6%)	16 (50.0%)	10 (31.3%)	1 (3.1%)
Female (n= 57)	2 (3.5%)	34 (59.6%)	18 (31/6%)	3 (5.3%)

Overall (n=89)	7 (7.9%)	50 (56.1%)	28 (31.5%)	4 (4.5%)

No statistically significant difference was found for diphtheria-specific antibodies between sexes, while there was significant difference between DTP booster status in Anti-diphtheria toxoid IgG level (Table 3).

Table 3. Difference of Anti-Diphtheria Toxoid IgG Level between Genders and DTP Booster Status. (n= 89)

		Median (min-max) (IU/mL)	**p-value***
Gender*	Male (n=32)	0.323 (0.001-1.886)	0.393
	Female (n=57)	0.183 (0.001-1.336)	
DTP booster at 18–24 months old**	DTP booster		
	Vaccinated (n=71)	0.348 (0.001–1.886)	
	Not vaccinated(n=18)	0.048 (0.001–0.747)	0.001

*Mann-Whitney test, Mean rank male 48.13, female 43.25.

**Mann-Whitney test. Mean rank vaccinated group: 50.92, unvaccinated group: 21.61.

Upon categorization according to the immunity status, there was a significant association between the history of first DTP booster at 18-24 months old. Among 89 participants, only 2 out of 18 children not receiving the first DTP booster at 18-24 months old had antibody protection for diphtheria (p=0.001, OR 27.5, 95% CI: 5.71 – 132.42; Table 4).

Table 4. Diphtheria Immunity According to The History of First DTP Booster Vaccination Status at 18-24 Months Old

		Immunity level of Diphtheria		**p-value***
		Immunity protection (Anti Diphtheria IgG≥ 0.10 IU/mL).	Susceptible (Anti Diphtheria IgG < 0.10 IU / mL)	
DTP-booster vaccination status at 18-24 months old	Vaccinated at 18-24 months old	55 (77.5%)	16 (22.5%)	0.001
	Not vaccinated at 18-24 months old	2 (11.1%)	16 (88.9%)	

*Chi-square test

Discussion

This study showed that children in the population have already missed the immunization, especially the booster vaccination at 18-24 months old. Data showed that COVID-19 has made an impact on life-saving immunization services around the world, putting millions of children at risk of diseases like diphtheria.[9] According to data collected by the WHO and UNICEF during the COVID-19 pandemic, more than half of the 129 countries (53%) reported severe disruptions in routine immunization in

childhood, including DTP vaccination.[10,11] History showed that the outbreak of diphtheria happened following the decreased coverage of DTP vaccination. In Indonesia, the diphtheria outbreak in 2017 happened as the coverage of DTP vaccination decreased.[2] In accordance with the Expanded Program on Immunization (EPI) recommendation, the Indonesian National Immunization schedule comprises primary vaccination with 3 doses of DTP at 2, 3, and 4 months old, followed by the first booster dose at the age of 18–24 months old.[12] Our study suggest there will be a need for a comprehensive policy to provide immunization access and immunity gaps in children.

In this study, 57 children (64%) were observed to have immune protection from diphtheria. Serological surveys are usually performed to determine the duration of immunity after primary and booster vaccinations and recommend strategies to reduce immunity gaps.[13] However, serological surveys are seldom conducted in low-income and middle-income countries because of resource constraints and restricted access to relevant clinical laboratories. For diphtheria, assay commonly used to determine the antibody level of protection is the anti-diphtheria toxoid IgG Enzyme-Linked Immunosorbent Assay. This is comparable with the survey in Vietnam, showing that 68% of children aged 0 – 5 years old had immunity against diphtheria.[8] Our immunity data of diphtheria in children is still better compared to the one in Malaysia,[14] showing that only 42.5% of children aged 5 to 6 years old had protection against diphtheria, and to the one in India, reporting that only 29.7% children aged 5-17 years old were immune to diphtheria.[15]

Our study supports that first and second DTP boosters are paramount to maintaining immunity among pre-school-age and school-age children against Diphtheria.

Conclusions

Diphtheria booster vaccination at 18 – 24 months old is crucial as it minimizes the decline in humoral immunity against diphtheria due to primary vaccination among pre-school children.

Competing Interests

There were no conflicts of interests in this study.

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