JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

The evaluation of anti-diphtheria toxoid antibodies after 5 years with the primary Tetanus-Diphtheria vaccine in persons aged 6-25 years in Kon Tum, Vietnam: a follow-up study

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Abstract

Introduction: Diphtheria is a serious infectious disease although being vaccine-preventable. 5-year follow-up study aimed to evaluate the persistence of IgG antibodies to diphtheria toxoid after Tetanus-diphtheria primary immunization for persons aged 6-25 years in Kon Tum, Viet Nam.

Methodology: Blood samples were obtained from 128 healthy persons aged 6-25 years collected in 2021. Samples were tested for diphtheria toxoid antibodies by commercial Anti-Diphtheria Toxoid IgG Enzyme-Linked Immunosorbent Assay (ELISA).

Results: One month after Td primary vaccination, 92.2% of participants were fully protected against diphtheria (> 0.1 IU/mL). GMCs increased from 0.04 to 0.91 IU/mL at one month, which were maintained in nearly 90% of vaccinees at 5 years. Among children aged 6-10 years, 97.3% had full protection at 1 month after primary vaccination, maintained in 94.6% at 5 years. Among adolescents and adults, 81.0% to 98.5% had full protection at 1 month after vaccination, which were maintained from 72.7% to 96.7% at 5 years. Although, the antibodies slightly declined approximately 5 years post-vaccination, but remained several-fold higher than pre-vaccination.

Conclusions: Td vaccine provides long-lasting protective antibodies against diphtheria. These data may inform discussion of the need for Td booster vaccinations among high-risk persons.

Key words: Diphtheria; tetanus-diphtheria vaccine; antibodies; seroprevalence; Kon Tum; Vietnam.

J Infect Dev Ctries 2025; 19(1):22-27. doi:10.3855/jidc.19390

(Received 12 October 2023 – Accepted 08 August 2024)

Introduction

Diphtheria is a highly contagious infectious respiratory disease which, although vaccine-preventable, remains to date a global public health concern. A total of 4490 diphtheria cases were reported worldwide in 2013, most of the diphtheria cases were in developing countries [1]. In Asia, diphtheria outbreaks have still been reported in Thailand [2], Lao PDR [3], and Vietnam [4].

In Vietnam, a total of 337 diphtheria cases were reported in period from 2016 to 2021 (13 cases in 2016, 21 cases in 2017, 13 cases in 2018, 53 cases in 2019, and 237 cases in 2022) [5]. Kon Tum is one of the remote mountain provinces belonging to the Central Highlands region of Vietnam where diphtheria is still the public health concern with 53 confirmed cases and 2 deaths between 2016 and 2020. The majority of diphtheria cases were group aged 6-15 years (48.0%), followed by 16-25 years; 26-45 years, and 0-5 years (12.0%) with rate being 24.0%; 14.0%, and 12.0%; respectively (unpublished paper).

Our primary survey on immunization in 2016 showed that the coverage percentage of diphtheria vaccination in Kon Plong was low [6]. The World Health Organization (WHO) recommends immunization of at least 3 doses of dT or Td to people without receiving any diphtheria dose or with an unknown history of diphtheria vaccination to provide protection [7]. Therefore, a vaccination campaign with 3 doses of Tetanus-diphtheria (Td) vaccine was given to all persons aged 6-25 years who had a high risk of diphtheria disease. The diphtheria-specific antibodies in persons aged 6-25 years were assessed at one month after a Td primary vaccination. However, information regarding the long-term persistence of antibodies after 5 years following vaccination with Td is important as well as the long-lasting protection of vaccine-induced immunological response is critical to help the

Table 1. Humoral immunity in the study population.

Sample	Susceptibility < 0.01 IU/mL			Basic protection 0.01-0.1 IU/mL			Full protection > 0.1 IU/mL			GMC (IU/mL)	95% CI
	N	%	95% CI	N	%	95% CI	N	%	95% CI		
Pre-vaccination	64	50.0	41.0-58.9	16	12.5	7.3-19.5	48	37.5	29.1-46.5	0.04	0.03-0.05
Post-vaccination at 1 month	6	4.7	1.7-9.9	4	3.1	0.8-7.8	118	92.2	86.2-96.2	0.91	0.69-1.20
Post-vaccination at 5 years	10	7.8	3.8-13.9	3	2.3	0.5-6.7	115	89.8	83.3-94.5	0.67	0.51-0.87

appropriate policy-making on the recommended time interval between booster vaccinations.

The study aimed to evaluate antibody persistence against diphtheria at 5 years following Td vaccination in Kon Tum province of the Central Highlands, Vietnam.

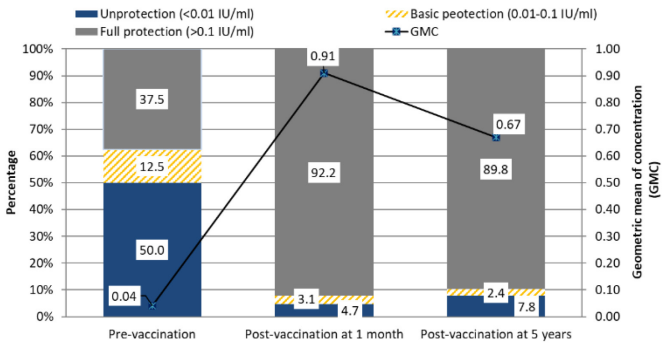
Methodology

Study design and sampling

In this longitudinal study during the period from June 2016 to November 2021. Participants studied in this long-term serology follow-up were those from an initial study in Kon Tum in 2016. Adolescents and adults aged 6 through 25 years, who received tetanus-diphtheria (Td) vaccine in 2016 were selected. Serum samples were collected at pre- and 3-month post-vaccination from participants who agreed to be contacted to provide additional blood samples after 5 years onward. They were subsequently contacted by health workers and via phone at approximately 5 years of post-vaccination to collect blood samples for serology testing. Participants were excluded from the study if they received any vaccine containing components of diphtheria antigens after the initial study vaccination or were clinically diagnosed or laboratory confirmed to be diphtheria infection.

Subjects were divided into age groups followed as 6–10 years, 11-15 years, 16–20 years, and 21–25 years old. Serum samples were frozen and stored at minus 20 °C during 72 hours before transportation to the laboratory of Tay Nguyen Institute of Hygiene and Epidemiology and stored at minus 80 °C until antibody testing was performed.

Figure 1. The distribution of diphtheria humoral immunity at pre- and post-vaccination.



Enzyme-linked assay

For determination of anti-diphtheria toxoid IgG antibodies, a commercial Anti-Diphtheria Toxoid IgG Enzyme-Linked Immunosorbent Assay Kit (ELISA) (Euroimmun, Germany) was used. WHO guidelines and interpret results the antibody levels < 0.01 IU/mL as not protective against infection (susceptibility), 0.01 to 0.099 IU/mL as basic protection, and > 0.1 IU/mL as full protection against diphtheria [8].

IgG anti-diphtheria toxoid antibodies was expressed as the geometric mean concentrations (GMC) with 95% confidence interval (95% CI). For the calculation of GMC, the undetectable values were excluded.

Ethical approval

This study was approved by the Ethical Committee of Kon Tum Department of Health, Vietnam (Approval number: 189a/QD-SYT, dated 6th May 2016). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All participants and their caregivers had provided written informed consent and assent, as appropriate, prior to study enrollment.

Statistical Analysis

The data was presented as mean ± standard deviation (SD). Geometric mean concentrations (GMCs) were calculated with associated 2-sided 95% confidence intervals (CIs). The chi-square test was used to verify the association between participant characteristics variables and seroprevalence, while Student tests or one-way ANOVA test could be used for comparison of GMCs among the analysed groups. A *p* value of 5% or lower was considered to be statistically significant. The anti-diphtheria anti-antibody seroprotection were analysed with multiple logistic regression. Statistics were performed using SPSS version 22 (IBM, USA).

Results

In total, 128 participants (42 males and 86 females) were included in the study. The mean age of years was 13.7 (± 5.5).

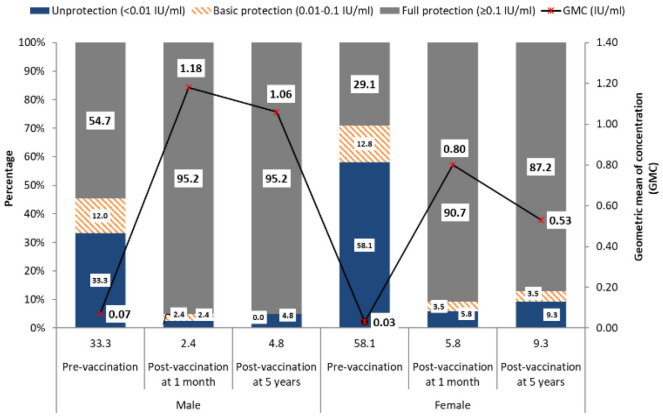
In a total of 128 participants, 50% of persons had no diphtheria antibody levels < 0.01 IU/mL prior to the receipt of Td vaccine). One month after vaccination, 92.2% of persons had diphtheria antibody levels ≥ 0.1 IU/mL; this proportion decreased slightly to 89.8% at 5 years follow-up (Table 1 and Figure 1).

Among males, 95.2% had full protection against diphtheria (≥ 0.1 IU/mL) at 1 month after vaccination and constant 95.2% at 5 years after vaccination. The full protection rates for diphtheria declined in females from 90.7% at month 1 to 87.2% at year 5) (Figure 2).

At 1 month after vaccination, the GMC of diphtheria antibodies increased compared with baseline by 15.5-fold in males (1.18 IU/mL, 95% CI: 0.81-1.74) and by 2.6-fold in females (0.80 IU/mL, 95% CI: 0.55-1.16). Although antibody levels subsequently slightly decreased over the 5-year follow-up period, they remained higher than pre-vaccination levels for diphtheria in all persons (Figure 2).

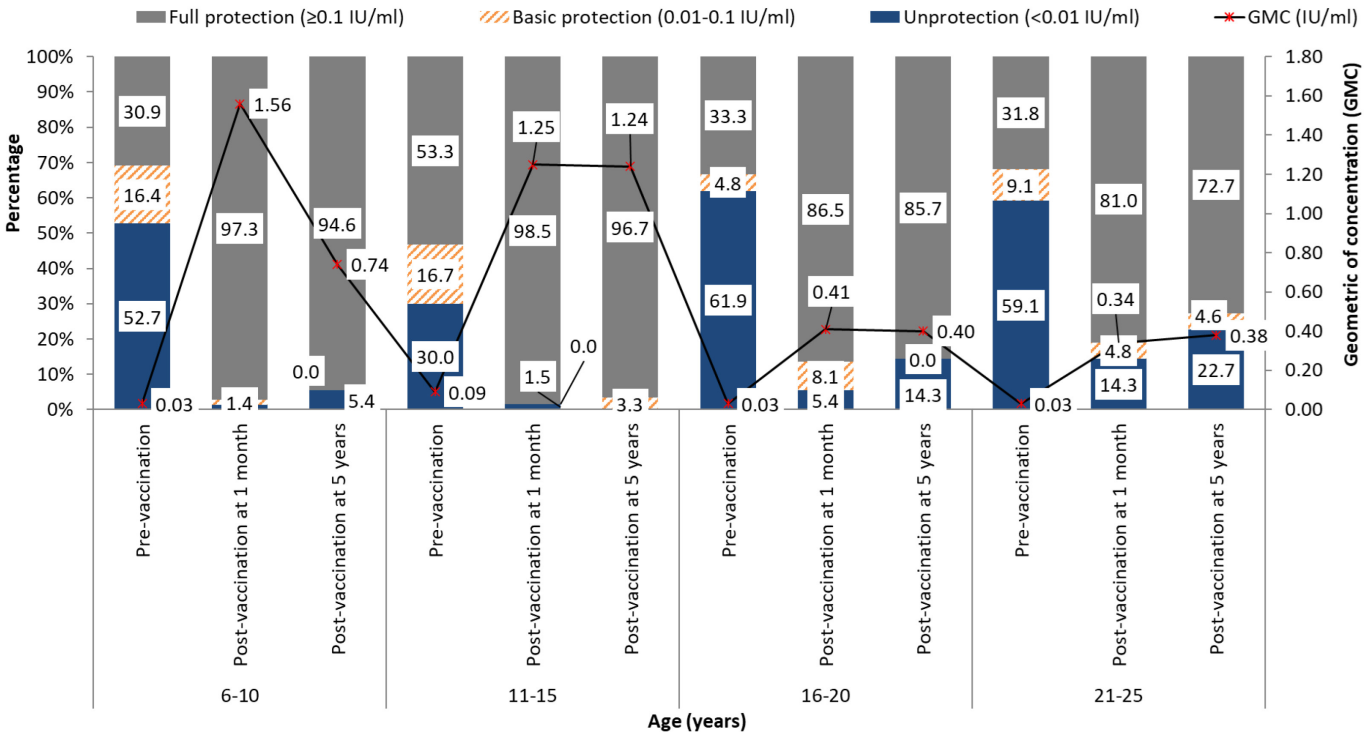
Overall, 92.2% of persons had full protection against diphtheria (antibody levels ≥ 0.1 IU/mL) at 1 month after vaccination. Of these, the highest full protection rate was observed in the groups aged 10-11 years (97.3%)) and the lowest protection percentage

Figure 2. Distribution of diphtheria humoral immunity by gender.



was observed in the groups aged 21–25 years (81.0%). Figure 3 showed that 97.3% of full protection for the group aged 6–10 years at 1 month after vaccination decreased to 94.6% after 5 years of vaccination. The proportion of individuals aged 11-16 years fully protected against diphtheria reached 98.5% one month after vaccination and maintained a high protection rate after 5 years of follow-up (96.7%). A similar decline was observed in aged 16-20 and 21-25 years. 86.5% and 81.0% of individuals aged 16–20 and 21-25 years were regarded as fully protected at 1 month after vaccination decreased to 85.7% and 72.7% after 5 years of vaccination, respectively (Figure 3).

Figure 3. Distribution of diphtheria humoral immunity by age group.



The overall geometric mean antibody concentration (GMC) of diphtheria toxin antibody at 1 month after vaccination for the groups aged 6–10, 11-15, 16–20, and 21–25 years reached 1.56 (95% CI: 1.27-1.92), 1.25 (95% CI: 0.88-1.77), 0.41 (95% CI: 0.16-1.02), and 0.33 (95% CI: 0.11-0.98) IU/mL; respectively compared to baseline. After 5 years of follow-up vaccination, GMCs maintained above protective level for all age groups (Figure 3).

In this study, a statistically significant difference in the fluctuation of antibody toxoid levels against diphtheria after 5 years of vaccination was found according to age groups. In contrast, no significant difference in the fluctuation of antibody toxoid levels against diphtheria after 5 years of vaccination was found according to gender, or ethnicity (Table 2). The highest antibody decrease rate was aged 6-10 years (83.6%) with OR = 7.4 (95% CI: 2.2-22.4), followed by 16-24 years (57.1%) with OR = 1.9 (95% CI: 0.6-6.5) compared to aged 21-25 years; while the lowest rate was aged 21-25 years (40.9%) ($p < 0.001$). The antibody decline was identified in 54.8% of males and 67.4% of females ($p > 0.05$). The proportion of antibody decrease was detected in 80.0% of the people who were Kinh, while this rate was only 62.2% in people who were others. However, no statistically significant difference was found ($p > 0.05$) (Table 2).

Discussion

In Vietnam, Expanded Program on Immunization (EPI) was first introduced in 1981 with the support of WHO and UNICEF [9]. Since 2009, Vietnam has vaccinated a DPT-VGB-Hib (Diphtheria-Tetanus Toxoids–whole cell Pertussis, Hepatitis B and Hemophilus influenzae) for infants aged 2, 3 and 4 months, DTP vaccine for children aged from 18 to 24 months and then a Tetanus diphtheria (Td) vaccine booster is recommended every 10 years for adults to maintain life-long protection

The importance of diphtheria vaccination was to reduce the growing burden of diphtheria among adolescents and adults and to reduce the risks to the lowest level of the transmission of the disease to children. In 2016, a diphtheria outbreak occurred with 3 confirmed cases, and 1 death in KonPlong district, Kon Tum province of Vietnam. Our primary survey on immunization showed that the coverage percentage of diphtheria vaccination in Kon Plong was low and up to 47.5% of persons aged 6-25 years were unprotective of diphtheria [10]. Adolescents and adults who have never been vaccinated a diphtheria-toxoid vaccine or unknown history of vaccination are recommended to receive 3 doses of DT or Td vaccine to provide protection [7]. During the 5-year follow-up, the percentage of participants with seroprotective levels decreased only slightly up compared to levels one-month post-vaccination and both the seroprotective

Table 2. Univariate and multiple logistic regression analysis of variables for diphtheria antibodies at 5 years post-vaccination.

(a) Univariate logistic regression analysis

Characteristics	Decline of antibody level (n = 81)	No change of antibody level (n = 47)	OR (95% CI)	$p^{\#}$ value
Gender				
Male (Ref)	23 (54.8%)	19 (45.2%)	-	-
Female	58 (67.4%)	28 (32.6%)	1.7 (0.8-3.6)	0.16
Age group				
6-10	46 (83.6%)	9 (16.4%)	7.4 (2.4-22.4)	**< 0.001**
11-15	14 (46.7%)	16 (53.3%)	1.2 (0.4-3.8)	0.68
16-20	12 (57.1%)	9 (42.9%)	1.9 (0.6-6.5)	0.29
21-25 (Ref)	9 (40.9%)	13 (59.1%)	-	-
Ethnicity				
Kinh (Ref)	4 (80.0%)	1 (20.0%)	2.4 (0.2-22.0)	0.442
Others	77 (62.6%)	46 (37.4%)	-	-

(b) Multiple Logistic Regression Analysis

Characteristics	Fluctuation of antibody toxoid levels against diphtheria post 5 years vaccination		
	OR	95% CI	*p* value*
Gender			
Male *(*Ref*)*	-	-	
Female	2.3	1.0-5.5	0.56
Ethnicity			
Kinh *(*Ref*)*	-	-	
Others	2.5	0.2-28.9	0.45
Age groups			
6-10	9.3	2.9-29.8	**< 0.001**
11-15	4.7	1.9-11.2	0.46
16-20	1.5	0.5-4.9	0.20
21-25 (Ref)	-	-	-

*Logistic regression analysis, main effects model. All variables are listed which attained a statistical significance of $p < 0.05$, #Chi-Square test.

rates and antibody GMCs were higher at years 5 than pre-vaccination levels. This extended follow-up study showed that toxoid-specific IgG antibodies against diphtheria persisted at elevated levels at 5 years after three primary doses of Td vaccine in children, adolescents, and adults. In Vietnam, the current EPI has been running a Td booster dose for individuals who have been vaccinated during childhood and then every 10 years.

In this study, seroprotective levels for diphtheria were still observed for 98% of participants up to 5 years post-vaccination with three primary doses of Td. This study data are in line with observations in young Belgian adults aged 20–28 years, with all participants maintaining antibody concentrations of 0.1 IU/mL for diphtheria 10 years after receiving Tdap vaccine [11]. The decline in seroprotection levels over time was found to increase with age and 10% and 45% of persons > 60 years old were not protected from diphtheria after 5 years [12]. Previous studies demonstrated that decline was also observed for diphtheria over a 5-10-year period in adults receiving a Tdap booster vaccination than in adolescents [13-16].

The WHO shows that 90% vaccine coverage in children and 75% in adults was adequate to establish herd immunity [17]. The risk of diphtheria outbreaks is heightened among populations with an immunity gap in adults and a low coverage of vaccine in children and adolescents [18], [19]. In addition, asymptomatic adult carriers also continue to put on a threat to susceptible children [7]. As a means of controlling potential diphtheria outbreaks, immunization coverage rates should be increased among at-risk groups.

In Vietnam, booster doses in adults are recommended every 10 years. Kon Tum is the mountainous and remote province belonging to the Central Highlands of Vietnam where ethnic minorities are a majority. The communes, villages, and districts in Kon Tum are very remote areas, therefore, access difficult to monitor and vaccinate target populations (both children and adults) for diphtheria booster vaccination.

This study has a few limitations. Firstly, we excluded known immunocompromised persons from participating in the study, this may result in underestimation of the susceptible proportion. Firstly, we do not know if participants have been exposed to *C. diphtheriae* over the 5 years of follow-up, which may have boosted antibody responses.

In conclusion, Td vaccine provides long-lasting protective immune responses against diphtheria. These data may inform discussion of the need for repeat Td booster vaccinations among high-risk persons.

Acknowledgements

We would like to thank all participants for their contribution to the study

Authors' contributions

TVL conceived the idea and designed the study; TVL, VTTN, QHN, TTTN, SDT, collected the data; TVL, CCV analyzed the data and drafted the manuscript. All authors commented the paper and approved the final manuscript.

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Conflict of interests

No conflict of interests is declared.

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