Antidiphtheria Antibody of Patients and Carriers Several Years after the Illness in Indonesia

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Abstract

Diphtheria is a lethal disease and toxin is the most important instrument of pathogenicity. Anti-diphtheria antibody plays a role in determining someone to be healthy, carriers, or be ill. Diphtheria infection will not provide a sufficient antibody level to the patient few months or years after. This study aimed to determine anti-diphtheria antibody level of individuals several years after someone being patients or carriers.

The participants of this cross-sectional study were all diphtheria carriers and patients aged ≤ 18 years in East Java Province Indonesia from the period of 2011-2015. The record was obtained from East Java Provincial Health Office. Subjects were visited, interviewed, and underwent physical examinations. Blood samples were obtained and the anti-diphtheria antibody level was determined using the Vero cell method. The result was then modified using WHO criteria. Data analysis used Mann-Whitney U, Kruskal-Wallis, and Chi-Square tests as appropriate with $p<0.05$ and 95% confidence interval considered as significant.

Among 25 carriers and 88 patients from 21 districts in the study, mostly above five-year-olds, only 11% carriers and 6% patients received three times immunization after the period of illness, indicating that the follow up by health care officers was not satisfactory. The antibody levels of the patients were significantly different from the carriers along with a prevalence ratio of 1.26 if the antibody was at a susceptible level. There were 8% carriers and 20% patients in the susceptible group.

In conclusion, six months to 3.5 years after having the illness, diphtheria patients remained to have lower anti-diphtheria antibody levels as compared to the carriers.

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Introduction

Diphtheria, caused by toxigenic *Corynebacterium diphtheriae*, is considered as a very dangerous disease.[1,2] Although in most developed countries this disease has already been eliminated, some parts of the world still have serious problem.[1,3,4-6] Since 2011, the incidence of diphtheria in Indonesia has been very high. Some certain provinces declared outbreak states. The most poorly affected area was East Java (with a population of 35 million) located in the eastern part of Java Island. In 2012, this province had almost one thousands clinical cases[3]. Culturally two tribes live in East Java. The Madurese tribe dominates the northern part of the province; meanwhile, at the other part, the predominant tribe is the Javanese. Much effort and some studies have been put forward to solve this diphtheria outbreak. However, until 2017 the problem continues.

The toxin is a primary instrument of pathogenicity in diphtheria infection.[2,7,8] Absorption of toxin can lead to death or severe damage.[1,8] Exposure to toxigenic *Corynebacterium diphtheriae* could end in 3 possibilities, healthy, patient, or carrier. Both patients and carriers have *C. diphtheriae* in the body, especially in their throat and or nose. A carrier tends to be a source of transmission and may also the possibility to be a patient. Carriers are the reservoir of the bacteria.[8,9,10] Immunity against diphtheria is one

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of the most important determinant factors for protection.[11,12] Many studies explore the antibody level during the period when someone got sick or carried *C. diphtheriae*.

Since diphtheria infection cannot raise sufficient level of permanent anti-diphtheria immunity,[8,9] health officers should follow every patient and provide them with diphtheria toxoid immunization. Completing the immunization will improve the antibody until it reaches the protective level. In Indonesia, there is no data about how the health officer follow all patients after hospitalization and immunize them. The objective of this study was to analyze the anti-diphtheria antibody of carriers and patients several months or years after the evidence of *C. diphtheriae* in their bodies. In this study, the antibody level had not been measured during the illness or while in a carrier state.

Materials and methods

Study Population and Samples

The population of this study was all diphtheria carriers and patients with the aged of ≤ 18 years living in East Java. This population has been identified during this outbreak, since 2011 until 2015. We planned to recruit all samples (total sampling). The data of diphtheria carriers and patients were kept in East Java Provincial Health Office. Data about culture results of nasal and throat swab were listed at Main Health Laboratory (Balai Besar Laboratorium Kesehatan=BBLK) Surabaya, one of the national reference laboratory for diphtheria in Indonesia. A person with clinical signs and symptoms of diphtheria such as fever, throat pain, and whitish pseudomembrane would be confirmed as diphtheria patients when the microbiology culture results found toxigenic *C. diphtheriae.* Once a patient was identified, health officer would seek all people who were in close contact with that patient, obtained specimens for culture, followed by a prophylactic antibiotic. Carriers were defined as persons with toxigenic *C. diphtheriae* in the throat and or nose, but without any clinical signs and symptoms of diphtheria. Until today there have been only one species, *C. diphtheriae*, included in the criteria for diphtheria infection in Indonesia. Identification of biotype was made according to WHO guidelines. Toxigenicity was determined by modified Elek test.

Data Collection

We identified the list at East Java Provincial Health Office and BBLK in Surabaya. All identified diphtheria carriers and patients across the province were visited. Their parents were interviewed and the child underwent a physical examination. Demographic data (age, sex, ethnicity) and immunization history were recorded. If the family had immunization card, the data from the card was used. Because of several resources limitations, we could not recheck the immunization data in the nearest community health center for validation. The house and surroundings were also examined, especially the size, density, ventilation, toilet facilities, and clean water sources. The blood samples were taken subsequently. Field data were collected until January 2016.

Blood Samples and Examination

Blood samples were sent to BBLK Surabaya on the same day or one day after. It was kept at proper temperature, after centrifugation. In the BBLK, sera were tested for reactivity to diphtheria toxin using in vitro Vero cell challenge neutralization assay. The Vero cell was from RVIM, The Netherlands. The semiquantitative results were interpreted using the WHO criteria: no immunity (<0.01 international unit (IU)/mL), basic level of protection (0.01-0.09 IU/mL), full immunity (0.1-1.0 IU/mL), and long-term protection (>1 IU/mL). Full or long-term protection levels of immunity were categorized as protective against infection[12,13].

Data Analysis

All data were processed using SPSS 17 (IBM Corp, New York). Statistical tests used Mann-Whitney U, Kruskal-Wallis, and Chi-Square tests as appropriate, with $p<0.05$ and 95% confidence interval considered as significant. Prevalence ratio was counted when there was a significant difference.

Ethical Clearance

This study was approved by Ethical Committee of Dr. Soetomo Hospital, Surabaya, Indonesia. All participants filled the informed consent before the data collection begun.

Results

Study Participants

From 173 children listed as diphtheria carriers and patients, 11 were already dead, leaving 35 carriers and 127 patients to be visited. Among 35 carriers, 2 refused to participate, 6 could not be located, and 2 others only accepted interviewed and physical examination but not the blood drawing. Among 127 patients, 7 refused to participate, 19 could not be located, and 4 only gave informed consent to be interviewed. From 97 blood samples of patients, 9 could not be processed in the laboratory. The total participants in this study were 25 carriers and 88 patients.

The participants came from 21 districts all over the province. Three other districts could not be included since the microbiology culture was not done in BBLK and their laboratory did not follow the WHO guidelines. Most subjects were from the period of 2012-2013 (89.5%), and above 5 year-olds. The subject characteristics were shown in table 1. There were no significant differences in all demographic characteristics between two groups.

Demographical Characteristics	Participants (N=101)		
	Carriers (N (%))	Patients (N (%))	
Age			
0–2-year-old	1 (3.7)	1 (1.0)	
≥2-5 year old	7 (25.9)	20 (19.8)	
≥ 5–12 year old	11 (40.7)	56 (55.4)	.82[a]
≥12–18 year old	8 (29.7)	24 (23.8)	
Sex			
Boys	11 (40.7)	58 (57.4)	.18[b]
Girls	16 (59.3)	43 (42.6)	
Recent Nutritional Status			
Undernutrition	5 (18.5)	19 (18.8)	
Good	12 (44.4)	70 (69.3)	.05[a]
Overweight and obesity	10 (37.1)	12 (11.9)	
Immunization Status			
Never	14 (51.9)	40 (39.6)	
Incomplete	9 (33.3)	25 (24.8)	.60[a]
Complete basic only	4 (14.8)	37 (35.6)	
Paternal Ethnicity			
Madurese	12 (44.4)	56 (55,4)	
Others	15 (55.6)	45 (44,6)	.42[b]
Immunization After Period of Illness			
3 times	3 (11.1)	7 (6.9)	
1-2 times	16 (59.3)	73 (72.3)	.63[a]
None	8 (29.6)	21 (20.8)	

Table 1. Demographical Characteristics of Diphtheria Carriers and Patients. Note: [a] Mann-Whitney U test; [b] Chi-square test

Antibody Level

The results of anti-diphtheria antibody level were shown in table 2, 3, and 4. There was a significant difference of antibody level between carriers and patients if the immunity level were divided into 2, susceptible and immune (Table 4). There was no difference if we use 4 categories as original WHO criteria (Table 2) The antibody level examination in this study took place 6

months until 3.5 years after the period of illness. For patients, these were antibodies some months or years after the illness, while for carriers, this was the antibody level months or years after they carried the bacteria. The median, minimum level, and maximum level for carriers and patients were the same, 0.512 IU/mL, zero, and 8.192 IU/mL, respectively. The median antibody level by year was shown in table 3.

Antibody Level (Iu/ML)	≤ 5 Year Old		>5 – 10 Year Old		>10–18 Year Old		Subtotal	
	Car Riers	Pa Tients	Car Riers	Pa Tients	Car Riers	Pa Tients	Car Riers	Pa Tients
No Immunity	0	0	0	2	1	9	1	11
Basic Level Of Protection	1	0	0	2	0	8	1	10
Full Immunity Long-Term Protection	0	1	4	9	8	15	12	25
	1	1	5	17	5	24	11	42
TOTAL	**2**	**2**	**9**	**30**	**14**	**56**	**25**	**88**

Table 2. Anti-diphtheria Antibody of Carriers and Patients According to WHO Criteria. *No immunity :* < 0,01 IU/mL; *Basic protection* : 0,01-<0,1 IU/mL; *Full immunity* : 0,1-1,0 IU/mL; *Long term protection* : >1,0 IU/mL.
The p value of Mann-Whitney U tests between carrier and patients for >5-10 year old, >10-18 year old, and all ages were 0.91, 0.74, and 0.76, respectively.

Years	Antibody Level (IU/mL)		
	Median	Min	Max
2012	0.512	0	8.192
2013	0.512	0	8.192
2014+2015	0.192	0	8.192

Table 3. Anti-diphtheria Antibody Level by Year.
Note: Kruskal Wallis test for the results by year; p=0.69

Age	Prevalence Ratio	95% CI	
		Lower	Upper
All ages	1.226	1.030	1.461
> 5–18 year-olds	1.278	1.097	1.488
> 5–10 year-olds	1.346	1.108	1.636
≥ 10–18 year-olds	1.259	1.038	1.527

Table 4. Prevalence Ratio between Carriers and Patients of Different Age Categories if the Immunity Level were Divided by Susceptible and Immune. Note : Susceptible = no immunity + basic level of protection; Immune = full immunity + long term protection

Discussion

Carriers are people who have bacteria but do not show any clinical signs and symptoms. They have important roles in disease transmission. Usually, someone will be a carrier only for short period.[9,10] It is known that during the Russian diphtheria outbreak the antibody anti-diphtheria level of carriers was higher than the patients.[9] The immunity will play a significant role in every diphtheria outbreak.[4,5,9,14,15] Carriers also have risk harboring other species of Corynebacterium.[16]

Indonesia has been suffering from diphtheria outbreak at least since 2011. The highest number of patients was in 2012, and until today this country has the second highest incidence of diphtheria cases in the world after India.[3] Not every part of the country had similar problems. The most affected province was East Java. This province was divided into two sociocultural areas. The northern part, dominated by the Madurese tribe, for such a long period showed worse outcome in almost all of the health related programs, including immunization coverage and outbreak of many diseases. In contrast, the southern and western part of the province, dominated by Javanese tribe, showed the good results of many programs.

Because of the limitations in facilities and experts, microbiology culture could only be performed in the two largest cities of the province; consequently, several remote areas could not be served well. The percentage of positive culture result was not good, either. The

data from East Java Provincial Health Office and BBLK Surabaya revealed only 6.5% positive results from more than 3000 recorded clinical cases. We identified 173 potential subjects for this study but at the end, only 113 were recruited.

The age distribution of carriers and patients were relatively indifferent. In contrast with the patients from Russia and several other countries,[17,18] most of the participants in this study were actually 5-18 year-olds. Around 80% of all patients were under 18 year-olds (Data from East Java Provincial Health Office). Similar reports came from Laos, Nicaragua, India, and Republic of Dominica.[4,5,6,15] The crude fatality rate in East Java was under 4%, compared with 32% in Dominican Republic[6] and 6.3% in Laos.[4]

There were no differences in immunization status between two groups. The reason was maybe that all subjects from both groups came from the same area. In the previous study, data from outbreak districts were not the same with those from different regions of the province, and statistically significant differences were noted[3]. Another possible reason was the small number of proven carriers. We assumed the number of true carriers was much larger than the record, but they were not detected by the health surveillance team. Survey on the relatively healthy children found 3% carriage rate[3]. Because of some difficulties in term of logistics and other resources we could not verify the data from the interview with an official record at the nearest community health center. It was proven that the differences between parents' memory and the official records were significant.[3,4,6]

Immunity in the endemic area can be developed by immunization or natural exposure to the bacteria,[3,4,9,11,19] although the mechanisms were possibly different.[12] People may not have received complete immunization but they still could show high immunity level. Indeed, this could be found in survivors only since some of those children could get severe diseases or even die because of the infection.

Indonesia introduced the first booster of diphtheria toxoid at 18 month-old recently. In the past, the first booster would be given at age 5-7 years. It was possible that before the booster the immunity level would be very low as shown in Islamabad and Laos.[4,20]

We did not have any data regarding antibody level of these participants when the cultures were done. Our examinations were performed approximately 6 months until 3.5 years after the period of illness. In all studies about immunity level, patients were shown having the lowest anti-diphtheria antibody.[4,5,9,21] Ideally, the antibody level should repeatedly be measured, starting from the time of illness, because the level would be different by time. One exposure to the toxigenic C. diphtheriae would raise the antibody for a short time only.[8,9] However, the results of antibody comparison based on the year of illness in this study were consistent.

In our study, 24% of patients and 8% of carrier did not reach the protective level of 0.1 IU/mL. The data indicated failure of the health officers to follow all patients and carriers for such a long time. This group did not receive adequate immunization after they were ill, as reflected in table 1 (immunization after positive culture). Moreover, some of this following immunization were done in outbreak response immunization programme and not be explicitly given specifically for specific patients. Surely these children with low-level antibody were at the similar risk to be reinfected and suffer from severe disease.[3,8]

Children with <0.1 IU/mL antibody level were at greater risk to be patients than carriers as indicated by the prevalence ratio. These results were consistent for all ages group in this study. Anti-diphtheria antibody is an almost absolute correlate of protection. It reflects the actual protection of our body against toxigenic *C. Diphtheriae.*[11,12] However, the original data which indicated the level of protection came from a study by J.Ipsen in the 1940s.[11] During the Russian outbreak, other studies were performed and showed similar results.[9]

Many studies proved that healthy people have the highest antibody anti-diphtheria compared with carriers group. The lowest level of antibody was found in patients.[9,22] When we compared our results with the previous study in the same area, it seemed the healthy children had the highest level of antibody,[3] which reconfirmed the results of the Russian study.

Clinically, it was very challenging to differentiate diphtheria patients and the carriers who got other bacterial infection. This fact could lead to pitfalls where in our study carriers might be misinterpreted as patients.[9,17] However, all diphtheria diagnosis during the outbreak was made by a medical doctor or even a specialist,

and this would hopefully reduce the misclassifications. Other weakness was the single time of antibody examination, but it was unavoidable considering our limitations in the province. This study was probably the first in the country using Vero cell method instead of ELISA to measure the antibody level. Vero cell is a WHO preference because of the instability of ELISA method.[23,24]

We suggest the result of this study be followed by additional immunization for the group with the lowest antibody level. Moreover, the follow up of carriers and patients should be better in the future. Other studies to ensure the quality of the vaccine and, more importantly, the cold chain, which often causes the failure of immunization are essential.[4,25]

Conclusion

In conclusion, in 6 months until 3.5 years after having harbored the bacteria, the antibody level of diphtheria patients was lower than the carriers. During the outbreak in Indonesia, many diphtheria carriers and patients were not sufficiently followed by the health officers.

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Declaration of Interest

The authors report no conflict of interests.

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