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A Fatal Diphtheria Case in a 4-Years-Old Child with Protective Diphtheria IgG Antibody in 2014

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ABSTRACT

Background: Basic Health Research Data 2010 show that the coverage of basic complete immunization was 48.8%. The lower of Immunization coverage will lead to vulnerability of diphtheria. A case of diphtheria was reported in the Regional General Hospital of Serang Banten, in a 4-years-old boy with symptoms of fever, difficulty swallowing, breathing and bullneck onset. The next day after sampling of throat swab, nose swab and blood, the patient died.
Objective. This study aims to identify the cause of death of patients with suspected diphtheria based on laboratory data.
Methods. Descriptive method by describing the events occurred. The sample cases and 2 close contacts were inoculated into the medium Cystine Tellurite Blood Agar (CTBA) and Blood Agar (BA). The toxigenic was detected by PCR, then the anti-IgG serology using reagents from AccuDiag TM.
Results. Culture of diphtheria cases showed the characteristics of diphtheria colonies and confirmed by microscopic evaluation, then biochemical tests by using API showed Corine>89.5% possibility of species *Corynebacterium diphtheriae* gravis type. Results of PCR showed positive results by the presence of tox gene (dtx) of *C. diphtheriae*. Serologic immune status showed the protective titers 1:18 IU/mL. (Protective titer > 0.1 IU / mL). The results of two close contacts were negative for diphtheria.
Conclusion. The death of patients could be caused by the faster activity of diphtheria toxin than the formation of toxin antibody to eliminate the toxin.

Keywords: Corynebacterium diphtheriae, IgG diphtheria, Serology

INTRODUCTION

Diphtheria is a frightening disease and also an old disease that has existed since Hippocrates. Hippocrates gave the first clinical picture of diphtheria in the 4th century BC. The modern clinical picture was made by Joost van Lom in 1560 and Baillou in 1576. They refer to the disease as 'quinsy' and 'croup'. The first epidemic of the disease occurred in Spain, known as 'morbus suffocans' or ‘garrotillo’ or suffocation mimicking garroting, a method of criminal execution carried out by Spain. From Spain the disease spreading to Italy in 1618 and known as the 'male in canna' or 'gullet diseases'.[1-4]

When it enters the body, diphtheria bacteria release toxins. This toxin will spread through the blood and can cause tissue damage throughout the body, especially the heart and nerves. Toxins usually attack certain nerves, such as nerves in the throat, so that it has difficulty swallowing in the first week due to the toxin produced. The toxin produced can also cause inflammation of the nerves of the arms and legs, resulting in weakness in the arms and legs. The damage can be severe, even leading to heart failure and sudden death. The combination of diphtheria treatment is done in two ways, the first is administration of ADS (anti diphtheria

serum) to neutralize the effects of toxins that have spread to the body and the second is the provision of antibiotics to kill diphtheria bacteria.[3-5]

The diphtheria prevention program is carried out with diphtheriae, pertussis, tetanus (DPT) immunization or diphtheriae, pertussis (DT) immunization. Both of these vaccines contain diphtheria toxoid which is an inactivated diphtheria toxin preparation. The aim of this immunization is expected to stimulate the formation of antibodies in the form of diphtheria anti-toxin. This anti-toxin will neutralize the toxin produced by C. *diphtheriae* bacteria, one of which contains diphtheria toxoid and not to kill diphtheria bacteria. While treatment for the elimination of bacteria in and contact diphtheria can be done by administering antibiotics.[4-6]

In line with this, Indonesia Riskesdas 2013 data states that coverage of basic immunization is still low at 53.8%.[6] The low complete basic immunization coverage lead to the concern of the emergence of diseases that cannot be prevented with immunization (PD3I). One such disease is diphtheria caused by the bacterial agent *Corynebacterium diphtheria.* These bacteria mainly attack the tonsils, pharynx, larynx, and nose, sometimes attack the mucous membrane or skin and sometimes the conjunctiva or vagina. The symptoms are painful throat, raised membrane lesions with cervical lymph nodes enlarged and tender. In moderate and severe cases characterized by swelling and edema in the neck with extensive membrane formation in the trachea and airway obstruction can occur which ultimately results in death.[1,6,7]

In Serang Province of Banten almost every month a case of diphtheria is reported. The latest data in 2014 based on the evidence of W1 form reported 34 reports of diphtheria outbreak events.[8] Based on the 2010 RISKESDAS data, complete basic immunization coverage is 48.8%.[7] Low immunization coverage will lead to vulnerability to diphtheria, as what occurred in July 8, 2014, when a diphtheria care was reported in General Hospital of Serang, in which a boy aged 4 years had symptoms of fever, difficulty swallowing, difficulty breathing and emergence of *bullneck.* On July 5, 2014 the patient went to the nearest private clinic and was given treatment, but there was no improvement. On 7 July 2014 the patient went to a different clinic but was immediately referred to the Serang Banten Public Hospital. On July 8, 2014 blood samples were taken for the fever panel and Anti IgG diphtheria serology. The patient also took a throat swab and nose, information obtained from the Local Health Office there was no history of DPT immunization, this was also confirmed by parents of the suspected diphtheria case. Therapy of erythromycin and anti-diphtheria serum (ADS) antibiotics was also given at that time. The next day, July 9, 2014 after sampling and contact, it was informed that the patient died. Sample examination was carried out at the Balitbangkes Bacteriology Laboratory by microscopy, culture and PCR. This study aims to determine the exact cause of death of diphtheria patients in Serang District General Hospital Banten.

METHOD

The method used in this research was descriptive method, which is a method that describes the events that occur. Sample of this study was patient with suspected diphtheria consisting of throat swabs sample, nasal swabs and blood was taken at the hospital isolation room of Serang Banten. The throat swab and nose swab samples were also taken from the contact, namely both parents. All samples were taken by the Center for Biomedical and Basic Health Technology officers, Balitbangkes Ministry of Health. The diphtheria suspect sample came from a diphtheria boy, 4 years old with symptoms of fever, sore throat, difficulty swallowing, pseudomembranous and bullnecks on his neck. No history of immunization was obtained. Another swab sample came from the 2 closest contacts, namely his parents. All swab samples were taken in isolation

room and inoculated into media *Cystine Tellurite Blood Agar* (CTBA) and *Blood Agar* (BA). Arriving in the laboratory immediately incubated at 37°C for 24-48 hours and then processed according to diphtheria examination standards.[1, 9-11] Biochemical tests were carried out using the Coryne API Biomeriux product.[12] Toxigenic examination was carried out by means of PCR,[1,9,10,13,] while serological examination of diphtheria IgG using reagents from AccuDiag [TM] Diphtheria IgG Elisa Kit.[14]

RESULTS

Cultures of samples from suspected case show the results of the characteristics of diphtheria colony in medium CTBA which appear black or gray because tellurite reduction. This was confirmed by microscopic results with a hammer-like appearance with a metachromatic granule at the tip (polarcele) and biochemical tests using the Coryne API showed> 89.5% possibility *of the* species *C Corynebacterium diphtheriae* Gravis type. PCR results to determine the toxigenic showed positive results which detected *toxin* gene (*dtx*) of *C. diphtheriae.* The results of serological suspicion of immune status using the ELISA technique showed a protective titer of 1.18 IU / mL (protective titer> 1.0 IU / mL). Culture results on both parents showed negative diphtheria results.

DISCUSSION

The number of diphtheria cases in 2014 in Indonesia was 396 cases with a total of 16 deaths so that the diphtheria CFR was 4.04%. Of the 22 provinces that reported diphtheria cases, the highest cases occurred in East Java, with 295 cases contributing 74% of the total cases. The number of diphtheria cases in East Java in 2014 decreased by half compared to 2013 which was 610 cases.[15]

Case data in Banten province in 2014 showed a total of 17 cases of diphtheria and only 4 cases had a history of vaccination, with 5 fatalities (CFR 29.41%). In 2018 diphtheria cases in Banten decreased to 5 cases, but all of them had no history of immunization and CFR reached 100%.[15.16,17]

Cases of diphtheria also occurs in other areas, such as in Tasikmalaya district where in 2005 and 2006 there were 55 cases and 15 (27%) of them died. Then in 2017 since January to November was recorded 593 cases of diphtheria, spread over 95 regencies and cities in 20 provinces throughout Indonesia, with a mortality rate of 32 cases. The following is data on diphtheria cases in Indonesia from 2014-2018 according to the World Health Organization.[9]

Table I. Number of Diphtheria Cases in Indonesia from 2014 to 2018

Year	Case Number
2014	430
2015	-
2016	342
2017	954
2018	1.026

Clinically, the case of the death of a 4-year-old boy in Serang Hospital, Banten was caused by diphtheria. The incubation period since *C. diphtheriae* infection is around 2-10 days. This was supported by the results of laboratory tests which discovered microbial culture with its *Corynebacterium diphtheriae* types Gravis. This type of gravis produces toxins that cause the death of the child. When bacteria infect the body, they release toxins or poisons. This toxin will spread through the blood and can cause tissue damage throughout the body, especially the heart and nerves. Toxins usually attack certain nerves, for example nerves in the throat. Patients have difficulty swallowing in the first week of toxin contamination. Between the third weeks to the sixth week, inflammation of the nerves in the arms and legs can occur, resulting in weakness in the arms and legs. Damage to the heart muscle (myocarditis) can occur at any time during the first week to the sixth week, is mild, appears as a mild abnormality on the ECG. However, the damage can be very severe, even causing heart failure and sudden death.

Recovery of the heart and nerves takes place slowly for weeks.[1,6,10] Based on animal experiments, the toxin excreted by diphtheria and passed into the body and causes damage to the tissue cells takes 10 hours. That is why in cases of suspected diphtheria ADS administration must be done immediately for patient therapy.[1,6,9,18]

Whereas, the results of diphtheria culture of the closest contact namely both parents showed negative results. An examination of the closest contacts is carried out to find the source of diphtheria transmission. An ideal source of infection should be searched for other closest contacts such as neighbors and friends, but this cannot be done because of limited sampling time.

Furthermore, serological tests for suspected diphtheria are carried out for a number of reasons, including assisting in clinical diagnosis, measurement of individual or population immunity and investigation of immune responses in individuals in certain regions. Measurement of serum antibodies to diphtheria toxin in cases before antitoxin administration may help to make a diagnosis especially if the culture results are negative. If the antibody level is low or under protective titer, it cannot neutralize the circulating toxin and if the antibody level is high the diphtheria attack should not become a systemic infection because the circulating toxin has been neutralized.[4,14,19]

While the immunological status of patients based on serological examination of diphtheria IgG Elisa showed a titer of 1.18 IU/mL. Titers <0.1 IU/mL indicate non-protective, titers ≥ 0.1 IU/mL indicate sufficient protection and titers>1.0 IU/mL indicate protective for long-term.[20] Test results for diphtheria suspected with a titer of 1.18 IU/mL indicate a very protective level for a long time, but how can someone with a protective immunity level but unable to protect from diphtheria toxin circulating in the patient's body and produced by C. *diphtheriae*. Information obtained from the family confirmed that the child has no history of immunization. Blood sampling for serologic was performed before the administration of cross-reactive ADS to eliminate false positive results. The emergence of diphtheria IgG antibodies in suspected diphtheria is likely due to exposure to *C. diphtheriae* 10-15 days before the patient is admitted to the hospital. Someone who is infected with *C. diphtheriae* with a small amount of toxin production will form natural antibodies.[4] Production of toxins stimulates antibody formation. The activity of the toxin in addition to stimulating antibody formation also results in tissue cell damage. When the number of antibodies formed is sufficient, some antibodies have neutralized, but some of the toxin activity first causes cell damage. Serological examination of diphtheria IgG shows protective concentration, but in reality, the patient dies and was not helped.

Toxin of C. *diphtheriae* gravis type is a type of toxin that has a fairly fast production time compared with other strain type. All diphtheria strains produce identical toxins and are able to colonize the throat. The difference in virulence between strains can be explained by differences in the ability to produce toxins (level and amount) and differences in average growth. Gravis strain has a *generation time* (in vitro) of 60 minutes, intermedius has a *generation time of* about 100 minutes and mythic about 180 minutes. In the throat (in vivo), faster growth allows the organism to spend iron supply more quickly, allowing faster and more toxic production.[9]

The nutritional status of diphtheria patients while in care was unknown, but sufferers in infants with severe malnutrition in diphtheria have a very close relationship. In infant's shortage of nutrition is often accompanied by iron (Fe) deficiency conditions in the body. This happens because toddlers lack energy and nutrient intake for a long time. Lack of iron (Fe) and also other nutrients such as protein causes a decrease in hemoglobin levels in the blood. Diphtheria toxin production expressed by *tox gene is* influenced by the presence of Fe,

the lack of Fe in the body will stimulate *tox gene* to produce toxins in large quantities. The toxin produced will work to damage cell tissue such as the heart and cause death.[18-2 2]

The administration of antibiotic therapy and ADS has been done by Serang District Hospital, but it cannot help the suspected diphtheria case. Death is thought to be caused by a delay in the provision of ADS because in general patients come to the hospital already at an advanced stage. Rapid working effect and production by *C. diphtheriae* causes tissue damage and results in death. Giving ADS and the presence of diphtheria IgG antibodies is not enough to neutralize toxins, the possibility of toxin activity on the host body is faster than administration of ADS and diphtheria IgG antibodies. Ideally, the ADS should be administered 3 days after the first onset (symptoms).

CONCLUSION

The case that occurred in Serang Banten Hospital is an extraordinary case with the discovery of positive *C. diphtheriae* gravis type, while the absence of a history of DPT immunization caused the severity of diphtheria. Diphtheria IgG antibodies that arise in the patient's body are a response due to the presence of toxins produced by bacteria, but diphtheria IgG antibodies that arise and administration of ADS cannot neutralize the toxin. Death may be caused by the working effect of diphtheria toxin that works faster compared to the body's immune response and ADS administration.

This case shows the important role of the government to continue to increase the basic immunization coverage program, which is still low in Banten Serang Province, and to disseminate to the public the importance of immunization early on. Rapid response measures and therapy from health workers also determine the success of diphtheria treatment.

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