Dengue virus infection amongst patients with acute febrile illness: clinical characteristics, molecular and serological profiles for vaccine trial and roll-out

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LIST OF ABBREVIATIONS

AFIRE	The Etiology of Acute Febrille Illness Requiring Hospitalization
CRF	Case Report Form
DENV	Dengue virus
DBD	Demam Berdarah Dengue
ELISA	Enzyme-Linked Immunosorbent Assay
ICF	Informed Consent Form
INA-RESPOND	Indonesia Research Partnership on Infectious DIseases
IRB	Institutional Review Board
IgM	Immunoglobulin M
IgG	Immunoglobulin G
NS-1	Dengue virus non structural-1 protein
PCR	Polymerase Chain Reaction
PRNT	Plaque Reduction Neutralization Test
RDT	Rapid Diagnostic Test
RSUD	Rumah Sakit Umum Daerah
RT	Research Team
SAE	Serious Adverse Event
SID	Subject Identification Number
SoC	Standard of Care
SOP	Standard Operating Procedure
SRB	Site Regulatory Binder
SAE	Kejadian Tidak Diinginkan yang Serius (KTDS)

I. BACKGROUND

Dengue is a significant public health problem in Indonesia, with 25.000-79.000 cases reported each year from 2013 to 2022. The disease is caused by four serotypes of Dengue virus (DENV-1, DENV-2, DENV-3, and DENV-4), transmitted primarily by the Aedes aegypti.

Comprehensive knowledge of the prevalence, immune response, and clinical characteristics associated with each serotype is essential for the development and implementation of effective dengue prevention and control strategies, especially in the context of vaccine development.

Previous studies have consistently shown that DENV-4 infections are relatively rare compared to other serotypes. A study in Vietnam found a low number of DENV-4 cases, illustrating the lower incidence of DENV-4 than other serotypes (Ta et al., 2019). A similar study in the Philippines, assessing the efficacy of a tetravalent dengue vaccine candidate (TAK-003) for DENV-4 in eight dengue endemic countries in Asia and Latin America was difficult due to the small number of DENV-4 infection cases, only 7 cases were virologically confirmed (Biswal et al., 2019).

A study on the prevalence and clinical aspects of DENV-1, DENV-2, DENV-3, and DENV-4 in Indonesia showed that DENV-4 was the least common circulating serotype (Harapan et al., 2019). A study conducted in many hospitals examining acute febrile illness in hospitalized patients (AFIRE), found that DENV-4 infection was less common among the cases studied (Utama et al., 2019). Interestingly, DENV-4 is often found in outpatients or during dengue outbreaks. A study in outpatients in West Java found that DENV-4 infection occurred as frequently as other serotypes, but most cases were not clinically typical (Kosasih et al., 2016). In the 2004 Jakarta outbreak, DENV-4 was the second most common serotype after DENV-3 (Suwandono et al., 2006). A study conducted during a dengue outbreak in Jember in 2019, has identified genetic factors associated with DENV-4, suggesting that a combination of selection pressure and changes in genetic mutations may contribute to the dominance of DENV-4 in East Java, Indonesia (Wardhani et al., 2023; Aryati et al., 2020).

One possible explanation for the low prevalence of DENV-4 is that most studies are hospital-based, which may not include untreated mild DENV-4 infections. Besides, NS-1 screening, which is used for detection and identification of DENV-4 cases, has low sensitivity, which further complicates accurate diagnosis (Mata et al., 2020; da Costa et al., 2014). In addition, the sensitivity of NS1 detection varies among secondary infections, with the highest sensitivity of DENV-3 (47.1%), followed by DENV-1 (40.9%), DENV-2 (30%), and DENV-4 (27%) (Kosasih et al., 2013). This is important as secondary

infections of DENV-2, DENV-3, and DENV-4 in Southeast Asia are commonly associated with severe clinical manifestations, indicating the potential for a significant health burden (Soo et al., 2016).

Considering the consequences of undiagnosed dengue infection, the inability to detect DENV-4 using NS1 rapid test may lead to misclassification of cases as non-dengue, resulting in different therapy/ management. This may adversely affect patient therapy, leading to delayed or inappropriate management potentially leading to prolonged illness or worsening of symptoms.

Overcoming the limitations of current diagnostic methods is critical to ensure quick and precise treatment for individuals infected with DENV-4. This is particularly relevant in the context of vaccine development and clinical trials, where accurate serotype identification is essential to evaluate vaccine efficacy and effectiveness. Improving diagnostic capabilities and awareness of the presence and potential impact of DENV-4 infection will contribute to more effective management and control strategies for dengue, ultimately reducing the burden of disease in affected populations. By addressing these issues, healthcare providers and researchers can improve patient prognosis and reduce the challenges posed by DENV-4 infection.

Tangerang, located in Banten province, Indonesia, has a high risk of dengue virus transmission due to its dense population and favorable environmental conditions for mosquito breeding. Despite vector control efforts, dengue remains a significant health challenge in the region. In 2022, as many as 50-100 dengue cases were reported in each public health center and more than 200 patients were hospitalized due to dengue infection.

Focused study in Tangerang, which includes hospitals and public health centers, is essential to obtain local data that can be used as a source of information for interventions that will improve a broader understanding of dengue epidemiology in Indonesia. Tangerang District Hospital is a referral hospital for all public health centers that has several national and multinational clinical studies. Including intervention studies, Tangerang District Hospital has a well-equipped infectious disease laboratory to conduct molecular and serological tests. Collaborating with INA-RESPOND lab, we are also able to conduct Plaque Reduction Neutralization Test (PRNT).

This prospective observational study in Tangerang aims to examine the prevalence, immunologic, virologic, and clinical aspects of dengue virus infection, especially DENV-4. The results of this study will improve our understanding of dengue epidemiology in Tangerang, Indonesia, the relationship between host factors, viral factors, and severity. This study will provide evidence-based information for dengue control, and

contribute to global efforts in developing an effective vaccine for all dengue virus serotypes.

The comprehensive screening efforts of this study will provide important data on other serotypes, enabling a holistic understanding of dengue infection and facilitating inter-serotype characterization. This study will have a significant impact on dengue research, public health policies, and global initiatives to combat this challenging disease.

II. OBJECTIVES

2.1. Primary objective

To determine the clinical characteristics, specific immune responses, and viral serotypes in patients with confirmed dengue virus infection in Tangerang District, Indonesia.

2.2. Secondary objectives

- To assess the prevalence of dengue virus infection among patients with acute fever
- To assess the prevalence of infection with DENV-4 and other serotypes as well as clinical characteristics, immunologic and virologic profiles important for vaccine trials and implementation
- To assess human and viral factors associated with severity of febrile illness due to dengue virus infection
- To increase the capacity of Tangerang District Hospital's Infectious Disease Laboratory/ INA RESPOND Reference Laboratory to perform plaque reduction neutralization test (PRNT).
- To identify other etiologies of fever in Tagerang district

III. CLINICAL HYPOTESIS

Dengue fever is a hyperendemic disease in Tangerang district, Indonesia.

DENV-4 infection is more frequently found at the primary health centers than at the hospital because the clinical symptoms are milder.

Several human and viral factors, such as age, nutrition status, immune status, serotypes, types of infections (primary/secondary) are associated with severity of symptoms.

IV. ETHICAL APPROVAL

This study will be conducted in accordance with ethical guidelines and obtained permission from the relevant institutions and the ethics committee of Tangerang District Hospital.

V. METHOD

5.1. Study design

This study is a prospective observational study that will be conducted in Tangerang Regency, Indonesia. In this study, data will be collected from patients who seek treatment at the Tangerang District Hospital, Kelapa Dua Public Health Center and Bojong Nangka Public Health Center in a one-year period from January 2024 to December 2024. Both public health centers have densely populated areas and have experience in conducting clinical research on COVID-19 vaccination.

Patients suspected of dengue infection will be screened using inclusion and exclusion criteria. Selected participants will be seen at the first visit as clinical data will be collected, routine hematology test and NS-1 RDT will be conducted. Blood specimens will be collected for further testing at Tangerang District Hospital's Infectious Disease Laboratory.

5.2. Inclusion and exclusion criteria

Inclusion criteria:

1. Patients age ≥ 4 years old with fever (≥ 37.5°C) or history of fever with antipyretic use, lasting less than or equal to 7 days
2. Presence at least one of the following dengue-like symptoms:
    - Headache
    - Retro-orbital pain
    - Nausea/ vomiting
    - Muscle, bone, or joint pain
    - Rash or bleeding manifestations
3. Patient or patient's guardian giving consent to participate in the study

Exclusion criteria:

1. Have a condition that may interfere with study procedures and compliance (based on doctor's assessment)
2. Suspected of focal infection

5.3. Study duration

The study will be conducted over a period of one year, from January 2024 to December 2024. Data will be collected from eligible participants visiting Tangerang District Hospital, Kelapa Dua Public Health Center and Bojong Nangka Public Health Center. Study period will be extended until it reaches the specified number.

5.4. Study procedure

- **Recruitment of participants**

  Patients who seek treatment at Tangerang District Hospital, Kelapa Dua Public Health Center and Bojong Nangka Public Health Center with dengue-like symptoms will be selected. All those who meet the enrollment criteria will be offered to participate in this study.

- ***Informed consent***

  Potential participants who meet the criteria, or their guardians, will be provided with detailed information about the study. Informed consent will be obtained from potential participants or their guardians prior to participating in the study.

- **Data collection**

  Data will be collected through structured interviews and medical record review. Information on demographic characteristics, clinical symptoms, medical history, laboratory results, therapy and previous dengue infection will be recorded in the case report form (CRF).

  Data on the number of patients presenting with fever to Tangerang District Hospital, Kelapa Dua Public Health Center and Bojong Nangka Public Health Center will be collected for use in identifying the proportion of dengue fever.

- **Data management**

  Participants will be anonymized to ensure participant confidentiality and the collected data will be stored in a locked cabinet with limited access.

5.5. Clinical data and specimen collection time

- **Hospitalized participants**

The data and specimens to be collected are data at the time of the first visit to the hospital and at the second visit. For participants with a positive NS1 RDT result at the first visit, second visit data will be collected when the participant is scheduled to be discharged from hospital inpatient care. For participants with a negative NS1 RDT result at the first visit, data for the second visit will be collected within 7-14 days of the first visit.

- **Participants from Public Health Centers**

Data and specimens to be collected are data and specimens at the time of the visit to the public health centers, while the second data and specimens are only taken as much as 25% of the number of participants of the first visit, with a window period of 7-14 days after the first visit. The determination of participants for the second visit is determined by study's team based on clinical judgment.

- **Clinical evaluation**

Participants will undergo a clinical assessment to evaluate the severity of the disease, including the presence of signs of severe clinical manifestations.

- **Type and amount of specimens to be taken**

- **Serum**

Serum obtained with a 4 mL yellow cap gel separator tube, all serum specimens obtained will be sent to Tangerang District Hospital's Infectious Disease Laboratory/INA-RESPOND lab for viral culture and PRNT.

- **Whole blood dan plasma**

Whole blood specimens in 3 mL purple cap EDTA tubes (adult) or 2 mL purple cap EDTA tubes (pediatric) will be used for hematology test, RDT IgM/IgG, and RDT-NS1 testing, the remaining plasma will be sent to Tangerang District Hospital's Infectious Disease Laboratory for ELISA and PCR testing.

5.6. Laboratory test

- Hematology

  Hematology test is conducted at PHCs and Tangerang District Hospital using an automatic hematology analyzer, and the results will be given to the doctor in charge as a basis for diagnosis and therapy.

- Antigen

  NS1 antigen will be tested at PHCs and Tangerang District Hospital using RDT, and the results will be provided to the doctor in charge as a basis for diagnosis and therapy. The results will be confirmed by NS1 antigen by ELISA.

- Molecular

  Blood from participants will be subjected to molecular testing. Polymerase Chain Reaction (PCR) to detect and confirm the presence of DENV,and identify dengue serotypes.

- Serology

  Enzyme-Linked Immunosorbent Assay (ELISA) will be used to detect dengue specific antibodies (IgM and IgG), Rapid Diagnostic Test (RDT) to detect IgM for acute dengue infection, and IgG to determine primary or secondary infection.
- Plaque Reduction Neutralization Test (PRNT)

  PRNT will also be performed to assess serotype-specific neutralizing antibodies in a subset of specimens (specimens from all severe cases and randomized mild cases as controls).

VI. STATISTICAL ANALYSIS AND SAMPLE SIZE JUSTIFICATION

6.1. Variable

Variables collected include age, gender, demographic characteristics, clinical symptoms, medical history, and history of previous dengue infection. The prevalence, immunologic and virologic characteristics, clinical features, and severity of DENV1-4 infection will also be recorded.

6.2. Statistical analysis

Statistical analysis will include descriptive statistics to summarize the demographic and clinical characteristics of participants. Prevalence estimates of DENV1-4 will be calculated with their respective confidence intervals. Prevalence, demographic and clinical comparisons between DENV-4 and other serotypes will be made using appropriate statistical tests, such as chi-square or Fisher's exact test.

6.3. Sample size

This study aims to collect data for one year. In accordance to the data reported in 2022, we will collect 100 dengue infection cases from each public health center and 200 cases from the Tangerang District Hospital.

It is expected that the Tangerang District Hospital, Kelapa Dua Public Health Center and Bojong Nangka Public Health Center can represent the population in Tangerang District.

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