INTRODUCTION

Dengue virus (DENV) infection causes febrile illness with wide clinical manifestations, ranging from asymptomatic or a mild flu-like syndrome known as classic dengue fever (DF) to a more severe form known as dengue hemorrhagic fever (DHF) and the potentially fatal dengue shock syndrome (DSS) (1). It threatens approximately 2.5 billion people living in endemic areas, with 50 million infections predicted to occur every year (2). Since the initial finding of DENV cases in Jakarta and Surabaya in 1968, DENV infection has become an endemic Arbovirus infection in Indonesia, especially in urban cities (3,4). According to an epidemiologic study, in 2017, there were 59,047 DHF cases and 444 DHF-associated deaths in Indonesia, with an incidence rate of 22.55 per 100,000 person-years and case fatality rate of 0.75% (5).

From 2004 to 2010, Indonesia ranked second worldwide in terms of dengue cases, accounting for an estimated USD 300 million every year (6). Indonesia has a significant prevalence of DENV infection. In 2006, 57% of DENV infections worldwide occurred in Indonesia, indicating that this disease is likely hyperendemic across most islands (7,8). In Indonesia, reporting DHF cases is mandatory within 72 h of diagnosis, based on the WHO 1997 Dengue Guidelines, to identify the case by a health center and public/private hospital (9).

DENV is an arthropod borne virus that is transmitted through Aedes aegypti and Aedes albopictus mosquitoes. DENV is a member of the Flavivirus family with an 11 kb genome size, under one open reading frame that encodes three structural (C, prM, and E) proteins and seven nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) proteins. Based on the E (envelope) gene variation, DENV is divided into four distinct serotypes that are antigenically different: DENV1–4 (10). These four serotypes were found to circulate in Indonesia (3). Laboratory diagnosis of DENV infection in hospitals and health services is limited to rapid NS1 dengue antigen detection; laboratory confirmation of dengue infection in Indonesia is also rare, and viral serotyping and genotyping are only undertaken by several institutions or universities on a project basis (11).
we used gold standards such as reverse transcription-infection. In this study, to confirm DENV infection, during the convalescence phase to confirm DENV the hospital and advised to return for blood extraction whereas NS-1 negative patients were discharged from Hospital, Jakarta for further observation and treatment, transferred immediately to Cipto Mangunkusumo DENV infection cases. NS-1 positive patients were conducted for hospitalized screening of suggested rapid test (Standard Diagnostic, Kyonggi, Korea). NS-1 for the presence of the NS-1 antigen using the NS-1 of the study, we drew the patients' blood and tested for the community who met the inclusion criteria. On day one visits by the medical staff in the community to locate significant effort, which involved daily door-to-door

| Table 1. Sequence of RT-PCR/serotyping primers |
|-----------------------------------------------|
| Primer’s code | Sequence (5’-3’) |
|----------------|------------------|
| Lan D1         | 5’-TCAATATGCTGAAACGCAGAGAAACCG-3’ |
| Lan D2         | 5’-TGCCACAAACAGTCAATGTCTTCAGGTC-3’ |
| TS1            | 5’-CGTTCAGTGATCCGGGGG-3’ |
| TS2            | 5’-CGCACAAGGGCCATGAACAG-3’ |
| TS3            | 5’-TAACATCATCATCATGAGACAGACG-3’ |
| TS4            | 5’-CTCTGTGTGCTTAAAACAAGAGA-3’ |

a limited number of studies have described DENV circulating serotypes in Indonesia. Thus, basic information about the circulating viral serotypes in the community can provide genomic epidemiological data for further development of vaccines or diagnostic approaches. In this study, we analyzed 102 serum samples of patients from the community suggested of having DENV infection using a molecular approach. This serotype distribution may be a predictive factor for future epidemiological analysis and is important for dynamic transmission models.

MATERIALS AND METHODS

Research design: In this study, we used a cohort design with consecutive sampling of active cases found in the community in Jakarta, Indonesia, from March 2010 to December 2011. The study was approved by the Research Ethical Committee Faculty of Medicine, Universitas Indonesia (No. 71/PT02.FK/ETIK/2009).

Subjects: Active surveillance was performed with significant effort, which involved daily door-to-door visits by the medical staff in the community to locate patients with fever of less than 48 h. Subjects of this study fulfilled the inclusion and exclusion criteria. The inclusion criteria were age > 14 years, fever with a temperature of 38°C for less than 48 h, provide informed consent, and fulfill the WHO criteria for dengue fever. The exclusion criteria were unwillingness to participate in the study, pregnancy, or underlying comorbidities.

DENV infection was confirmed in patients from the community who met the inclusion criteria. On day one of the study, we drew the patients’ blood and tested for the presence of the NS-1 antigen using the NS-1 rapid test (Standard Diagnostic, Kyonggi, Korea). NS-1 was conducted for hospitalized screening of suggested DENV infection cases. NS-1 positive patients were transferred immediately to Cipto Mangunkusumo Hospital, Jakarta for further observation and treatment, whereas NS-1 negative patients were discharged from the hospital and advised to return for blood extraction during the convalescence phase to confirm DENV infection. In this study, to confirm DENV infection, we used gold standards such as reverse transcription-polymerase chain reaction (RT-PCR) (Lanciotti et al., 2012, with slight modification of Dewi et al., 2014) (12,13), viral culture using the C6/36 cell line (Standard Operational Procedure, Department of Microbiology, FKUI), index value on IgG enzyme linked immunosorbert assay (ELISA) (IgG ELISA, Focus, Diagnostics, California, USA) or hemagglutination inhibition (HI) test (14). RT-PCR was also used to determine the DENV serotypes. HI test was performed to determine the type of infection, whether primary or secondary. Dengue infection with convalescent HI antibody titers less than 1280 was classified as primary infection (1). With respect to the IgG ELISA, the index value was calculated by dividing the specimen optical density (OD) by the mean of the calibrator’s OD. Specimens with an IgG index value > 1 were confirmed to be positive for DENV infection. Specimens with negative IgG results from the acute phase were classified as primary infection (15). These tests were performed at the Department of Microbiology, Faculty of Medicine.

Viral serotyping: A two-step RT-PCR was used to determine the serotype of DENV (12), with slight modification (13). DENV was determined as DENV-1, DENV-2, DENV-3, and DENV-4 when the RT-PCR products were 482 bp, 119 bp, 290 bp, and 392 bp, respectively (12). The first step PCR mixture (40 μL) contained 4 μL of 10× PCR buffer with 1.5 mM of MgCl₂, 0.8 μL of 10 μM Land D1 and Land D2 primers (Table 1), 0.8 μL of each 10 mM dNTP, 0.4 μL of SuperScript II RTase and Platinum DNA polymerase were purchased from Invitrogen (Carlsbad, CA, USA). Thermocycling (Applied Biosystem Programmable 9700 Thermal Cycler; Applied Biosystem, Foster City, CA, USA) was conducted as follows: 53°C for 30 min, 95°C for 5 min; 30 cycles at 95°C for 45 s, 60°C for 30 s, and 72°C for 90 s; and a final heating at 72°C for 7 min. The second PCR mixture (25 μL) contained 2.5 μL of 10× PCR buffer with 1.5 mM MgCl₂, 0.5 μL of 10 mM each dNTP, 0.15 μL of 5 U/μL Platinum Taq DNA polymerase, and 8 μL of RNA. SuperScript II RTase and Platinum Taq DNA polymerase were purchased from Invitrogen (Carlsbad, CA, USA). Thermocycling (Applied Biosystem Programmable 9700 Thermal Cycler) was conducted as follows: 53°C for 30 min, 95°C for 5 min; 30 cycles at 95°C for 45 s, 60°C for 30 s, and 72°C for 90 s; and a final heating at 72°C for 7 min. The second PCR mixture (25 μL) contained 2.5 μL of 10× PCR buffer with 1.5 mM MgCl₂, 0.5 μL of 10 mM each dNTP, 0.15 μL of 5 U/μL Platinum Taq DNA polymerase (Invitrogen), 1 μL of 10 μM D1, TS1, TS2, TS3, and TS4 primers (Table 1), and 2 μL of the first PCR product. Thermocycling (Applied Biosystem Programmable 9700 Thermal Cycler) was conducted as follows: 95°C for 5 min; 35 cycles at 95°C for 45 s, 60°C for 30 s, and 72°C for 60 s; and a final step at 72°C for 7 min. For specimens with PCR results showing co-infection with more than one serotype, we repeated PCR-II with primers for each serotype.

RESULTS

In this study, 102 patients with suggested DENV infection in the community of Jakarta were included. Patient characteristics were classified according to age,
sex, DENV infection status, and DENV serotype, as listed in Table 2. Briefly, 66.7% of the patients suggested of having dengue were positive for DENV infection, according to the gold standards. The age of the patients ranged from 14 to 23 years (50%), and there was no difference in gender distribution. The type of infection was mainly secondary infection (60.3%); DENV-2 was the predominant serotype (20.5%), followed by DENV-1, DENV-3, and DENV-4. Several mixed DENV infections were also found in 1–2.9% of cases. Viral culture using the C6/36 line had a low sensitivity, with only 12 (17.6%) testing positive among 68 confirmed DENV infections in this study. Among NS-1 negative patients, we found 2 patients with an index value > 1 and positive IgG results from the acute specimen using IgG ELISA. These patients were considered positive for secondary DENV infection.

The different disease severities of patients with positive RT-PCR results are shown in Table 2. In this community-based DENV study, the patients did not develop severe disease such as DSS (Table 3). In DENV-2 infected patients, 71.43% had mild disease. A similar result was found in DENV-4 infected patients (Table 3). In contrast, 68.75% of DENV-3 infected patients showed more severe disease with plasma leakage (DHF-I and DHF-II), which was also noted in
DISCUSSION

The aim of this study was to analyze the circulating DENV serotypes from 2010 to 2011 in a community in Jakarta. In addition to samples taken from hospitalized patients, in this study, medical staff members actively surveyed cases in the community by conducting door-to-door visits to locate patients with fever of less than 48 h, thus increasing the possibility of detecting the viral genome in the blood. This early detection resulted in establishing confirmed DENV infection in 66.7% of the subjects; thus, owing to this approach, patients could receive timely treatment and daily monitoring and evaluation, thereby preventing the development of severe dengue, specifically DSS. Early detection and appropriate management are important to reduce morbidity and mortality (16).

In this urban community setting of Jakarta, we found that DENV-2 was the predominant circulating serotype. Other community-based studies conducted in the urban area of Bandung during 2000–2002 (17) and recently in Jakarta in 2014 (7) also showed the predominance of DENV-2. Among the four community-based DENV studies in Indonesia, only one study, conducted in a community in Makassar during 2007–2010 (Table 4), showed a serotype other than DENV-2 to be the predominant (18). As summarized in Table 4, the first report of DENV-2 in Jakarta was during 1973–1974 and is the only report showing DENV-2 as the predominant serotype within this period (19). This finding is interesting because to the best of our knowledge, DENV-2 was not the predominant serotype in Jakarta or in other Indonesian cities in the later years (20–22).

Hospital-based studies showed that the predominant serotype was DENV-3 (6,8,23–34) followed by DENV-1 (35–41). The other serotypes were also found to be circulating, as shown in this study, which strengthens DENV endemicity in Indonesia. From these historical studies, we assume that infection with DENV-2 may result in less severe clinical manifestations than DENV-1 or DENV-3, which is commonly found in hospitalized patient samples. Our study showed that in DENV-2 infected patients, 71.43% developed mild disease. A similar result was found in DENV-4 infected individuals. Meanwhile, 68.75% of DENV-3 infected patients developed severe disease with plasma leakage (DHF-I and DHF-II). Commonly, Indonesian people visit hospitals when they have severe diseases (42). A study has shown that 60% of patients with severe disease visit the hospital to seek medical help (22). Although previous studies have shown various DENV serotype predominance depending on the study design,
season, and geography in Indonesia, our findings suggest that the most dominant serotype in the hospital and community settings are DENV-3 and DENV-2, respectively.

In comparison with community-based DENV studies, hospital-based DENV studies were more often conducted in Indonesia (up to 85.19%) (Table 4). DENV-4 was never the most dominant in both study designs. The dominant serotype in the hospital-based DENV study was DENV-3, followed by DENV-1 and DENV-2 at 47.83%, 13.04%, and 39.13%, respectively. In contrast, the serotype dominant in the community-based studies was DENV-2 (75%) (Table 4).

Although the correlation between DENV serotypes and disease severity is still unclear, several studies in other countries have shown that there is a tendency for this phenomenon to occur. In Singapore, DENV-1 has a more severe clinical manifestation than DENV-2 in adult patients (43). Meanwhile, a cross-sectional study in Brazil showed that severe DENV infection was found to be seven times more frequent among cases of DENV-2 than among cases of other serotypes (44). A meta-analysis in Southeast Asia (SEA) found that a primary infection with DENV-3 and secondary infection with DENV-2, DENV-3, and DENV-4 in SEA, as well as DENV-2 and DENV-3 infection in non-SEA regions, increased the risk of severe DENV infections (45).

The contribution of viral serotypes to different clinical findings was assumed to be correlated with the viral load. Higher peak DENV titers were associated with increased disease severity (46). In conclusion, this community-based study confirmed the distribution of multiple dengue serotypes in Jakarta, with DENV-2 being the predominant circulating serotype. Most patients acquired secondary dengue infection, and infection with DENV-2 tended to result in less severe disease than infection with DENV-3. Nevertheless, the current study found that minor viral discrepancy at the genotype level may also contribute to varied disease severity (47), which needs to be further explored in future studies.

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Conflict of interest None to declare.

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