IJID Regions 14 (2025) 100612

Contents lists available at ScienceDirect

IJID Regions

journal homepage: www.elsevier.com/locate/ijregi

Clinical and laboratory profiles of dengue infection in the hospitals in North Jakarta, Indonesia

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ARTICLE INFO

Keywords:
Dengue fever
Arbovirus
DENV serology test
Multiplex RT-qPCR
WHO guidelines for dengue

ABSTRACT

Objectives: Indonesia is one of the dengue endemic countries. The criteria for diagnosing dengue infection are based on World Health Organization (WHO) guidelines. Chikungunya and Zika virus infections have also been reported sporadically in Indonesia. This study aimed to evaluate the clinical features of patients with dengue in a hospital setting and investigate the potential for other arboviral infections in patients with fever.

Methods: This case-control study was conducted at two hospitals in North Jakarta between August 2023 and May 2024. Patients admitted with 3 or more days of fever without any proven cause of bacterial infection or autoimmune disease were recruited. The cases were those who tested positive for Dengue, Chikunguya, Zika, and West Nile viruses by multiplex reverse transcription-quantitative polymerase chain reaction, whereas the controls were those who tested negative. Questionnaires were used to collect the signs and symptoms. Blood tests were conducted using autoanalyzers. Chi-square and Student's *t*-tests were used for statistical analyses.

Results: Of the 135 respondents, 70 tested positive for dengue and two tested positive for chikungunya using multiplex reverse transcription-quantitative polymerase chain reaction. Having fever with two additional two signs and symptoms, as per the WHO guidelines, is predictive of dengue infection. Leukopenia and thrombocytopenia were significantly more common in dengue cases. An increase in hematocrit was inconclusive. Serum aspartate transaminase levels are also increased in patients with dengue. The dengue virus nonstructural protein-1 antigen test is the preferred point-of-care test for the diagnosis of dengue virus infections.

Conclusions: Our investigation confirmed that the WHO guidelines for diagnosing dengue are still applicable. The Chikungunya virus also circulates in Jakarta, and physicians should be aware of this.

Introduction

Dengue virus (DENV) is a *Flavivirus* that can be spread by mosquitoes, and, hence, also known as one of the arboviruses. The virus is transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes [1]. Dengue fever remains a global health problem, with more than 7.6 million cases and more than 3000 deaths reported by April 2024 [2]. Indonesia is still affected by the burden of dengue. The incidence rate of dengue hemorrhagic fever in Indonesia between 2013 and 2023 fluctuated from 24.8 to 78.9 per 100,000 population [3] As of the twenty-second week in June 2024, 119,709 cases with 777 mortalities were reported [4]. Hence, the Indonesian government designated dengue as a top priority disease and established a dashboard for dengue cases.

The management of dengue cases in Indonesia followed the standards outlined in the decree of the Ministry of Health No. HK.01.07/MENKES/2020, based on World Health Organization (WHO) guidelines. The guidelines that were composed in 2011 are mostly based on clinical characteristics and basic blood testing, i.e. thrombocytopenia and an increase in hematocrit [5]. Nevertheless, other diagnostic

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https://doi.org/10.1016/j.ijregi.2025.100612
Received 11 October 2024; Received in revised form 20 February 2025; Accepted 21 February 2025

methods available in Indonesia primarily use serologic tests such as immunoglobulin (Ig) M or IgG anti-dengue antibody tests and nonstructural protein-1 (NS-1) dengue antigen tests. Most of these tests use the principles of lateral flow immunoassays (LFIAs). These tests are frequently used in primary care settings owing to their low cost and rapid production of results. Other immunoassay methods, such as enzyme immunoassays and electrochemiluminescence immunoassays, are also available but usually more expensive and only available in a few laboratories [6].

Other important arboviruses are Chikungunya virus (CHIKV) and Zika virus (ZIKV). CHIKV infections have been reported sporadically in Indonesia. However, this tends to be disregarded by physicians who focus on dengue. A study in Indonesia reported a CHIKV prevalence of 3.7% in patients with fever in eight hospitals between 2013 and 2016 [7]. ZIKV infections have also been reported in Indonesia [8,9]. Although Singapore experienced a ZIKV infection outbreak during the second half of 2016 [10], there has been no report of an increase in ZIKV infections in Indonesia despite frequent traffic between both countries.

The high burden of DENV infection alerts the health system in Indonesia, but the infection of CHIKV and ZIKV does not receive much attention. CHIKV and ZIKV are also transmitted by *Aedes* spp.; therefore, they are also a potential threat to the Indonesian health system, as in other countries [1,11,12]. Although the clinical symptoms of CHIKV infection resemble those of DENV infection, arthralgia is a notable symptom of chikungunya fever [13].

Although controlling DENV infection has been the priority of the Indonesian government, dengue fever and its more severe forms, grades I-IV of dengue hemorrhagic fever, still pose a health problem, with a yearly surge of cases during the rainy seasons. During this time, accidentally accumulated rainwater in the reservoirs serves as a breeding ground for *Aedes* spp. mosquitoes, thus escalating the rate of transmission.

Serologic methods for the diagnosis of dengue have several limitations. Antibodies take time to develop and their presence can eliminate pathogens. IgM can be detected earlier, but IgG develops later. However, IgG may persist for an extended period and only indicates previous infections [14]; therefore, it has low sensitivity and specificity. The NS-1 dengue antigen is considered to possess higher specificity but relatively low sensitivity.

The polymerase chain reaction (PCR) detects nucleic acids via amplification. The reaction was specific because of the sequence of the forward and reverse primers used. For TaqMan quantitative PCR (qPCR), a probe was used to enhance specificity. This method emits fluorescence signals that can be detected by sensors and is used for quantitative comparisons. Because of this amplification, the assay was highly sensitive. TaqMan qPCR can be designed as a multiplex method to detect several target sequences, thereby enhancing its versatility for detecting several pathogens that exhibit similar symptoms. A reverse transcription (RT) step was added to synthesize complementary DNAs from RNA material, including from RNA viruses' genomics such as DENV, CHIKV, and ZIKV, which are then used for subsequent amplification. This method is known as RT-qPCR. Commercial kits for DENV RT-qPCR are available in Indonesia and usually in a multiplex form that detects DENV, CHIKV, and ZIKV simultaneously because of the same route of transmission and relatively similar symptoms.

This study aimed to examine the clinical characteristics of adult patients admitted to two referral hospitals with 3 days of fever without any proven bacterial infections or autoimmune diseases. A case-control approach was used, utilizing multiplex RT-qPCR as a diagnostic tool to categorize the respondents into case and control groups. Specifically, the focus was on the parameters related to the WHO guidelines 2011 to indicate their applicability to the current situation in Indonesia. We also examined the possibility that CHIKV and ZIKV are other sources of infection because of their similar clinical manifestations.

Materials and methods

Study design, data, and specimen collection

This case-control study was conducted between August 2023 and May 2024. This period included the rainy season when the incidence of dengue peaked. Respondents were patients who were admitted to adult wards in two hospitals: Atma Jaya Hospital (AJH), an intermediate referral academic hospital. Duta Indah Hospital (DIH), a first-entry referral hospital that receives patients directly from the primary clinics in the vicinity, was included to accommodate the national referral system. Both hospitals are in the Penjaringan subdistrict of North Jakarta. Respondent recruitment in AJH started in August 2023 and continued until May 2024, whereas recruitment in DIH started in February 2024 and continued until May 2024. Because of its status as an intermediate referral hospital, additional laboratory tests, including blood chemicals, are available for patients admitted to the AJH, if justifiable.

The study was scheduled to coincide with the rainy season in Jakarta, which typically concludes at the end of April. We used STATCALC from EPIINFO v7.2.6.0 (Center for Disease Control, USA) to calculate the required sample size, applying a 95% confidence level, 80% power, 12.4% of cases with exposure, and an odds ratio (OR) of 2.6 [15]. This calculation resulted in 70 cases and 70 controls as the recommended number of respondents.

All adult patients with age older than 18 years old, admitted to both hospitals, with fever (≥38°C) for 3 days or longer, without any evidence of bacterial or other source infections, were asked for their participation and informed consent. Data on age, sex, dwelling type, and clinical symptoms, including those mentioned in the WHO guidelines for DENV infection [14,16,17], were collected through questionnaire-guided interviews. The exclusion criteria for this study were patients already confirmed to have other causes of fever such as autoimmune diseases and bacterial infections. Respondents were considered cases if their RT-PCR results for DENV were positive. The other respondents were considered controls. This study did not introduce additional treatments to the cases or controls. According to hospital protocol, patients were administered intravenous physiologic solutions.

The blood specimens were collected from leftover K3-ethylenediaminetetraacetic acid blood of each patient, which were previously used for blood tests and had been stored at 4°C for a maximum of 24 hours. Hematologic profiles (hemoglobin, hematocrit, leukocyte count, differential count, and platelet count) were collected from the hospital information system. Both hospitals use automatic blood analyzers. The AJH uses Sysmex XN-550 and the DIH uses Mindray BC5380. The respective normal values of the analyzers were used as the cut-off values (COVs) for abnormalities. Several respondents from AJH had data on blood chemicals such as aspartate transaminase (AST), alanine transaminase (ALT), blood urea, and creatinine. If available, the data were also recorded. Some patients who visited AJH underwent DENV serologic tests as part of the referral data. The available tests include the LFIA combination tests for DENV IgM and IgG antibodies and the LFIA DENV NS-1 antigen test.

The ethylenediaminetetraacetic acid blood specimens were transported to the research laboratory in a cold chain. Upon arrival, the plasma was separated by centrifugation, aliquoted into 1.5 ml microtubes and stored at −80°C for further examination.

RNA extraction and RT-qPCR

All serum samples from the recruited respondents were subjected to RT-qPCR for arboviruses. RNA was extracted from plasma using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany, Cat. #52906) according to the manufacturer's protocol. Exogenous internal control was added to each sample to determine the appropriate RNA extraction process. RT-qPCR was performed using the Novaplex Tropical Fever Virus Assay Kit (Seegene, Seoul, South Korea, Cat. #RTF10208W), a multi-

plex RT-qPCR that detects CHIKV, DENV, ZIKV, and West Nile viruses (WNVs). RT-qPCR was performed using a CFX96 Touch (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. Nuclease-free water and a tropical fever virus–positive control provided by the manufacturer were used as negative and positive controls, respectively. The Seegene Viewer V3 application was used to analyze the RT-qPCR results. Samples were classified as positive for DENV, CHIKV, ZIKV, and/or WNV when the cycle threshold (Ct) of the samples was less than 45 and exhibited a sigmoid amplification curve.

Data management and analysis

All data were compiled using Microsoft Excel and subsequently exported to SPSS ver. 25 (IBM SPSS Statistics, Armonk, NY, USA). Descriptive analysis and statistical tests were performed to examine the correlation between clinical manifestations, blood profiles, and RT-qPCR results, using the chi-square test for categorical data and Student's *t*-test for continuous data. All the cases and controls had data on blood tests and the results of RT-qPCR. Not all respondents had data on blood chemicals and LFIA for dengue; therefore, the number to be analyzed for these parameters was different from the clinical features and blood counts. A value of $P < 0.05$ is considered statistically significant.

Ethical review and clearance

All patients were recruited during hospitalization. Informed consent was provided by the patients before being interviewed by a trained enumerator accompanied by attending physicians or nurses. Laboratory data and leftover specimens were collected from laboratories in each hospital. The study protocol was reviewed by the Internal Research Ethics Committee and granted clearance no. 21/05/KEP-FKIKUAJ/2023. The inclusion of DIH required amendment and was granted clearance No. 11/02/KEP-FKIKUAJ/2024.

Role of the funding source

The funding sources had no role in the study design, data analysis, data interpretation, writing of the report, decision to submit for publication, or any other aspects pertinent to the study.

Results

Demographic characteristics and RT-qPCR results of the respondents

A total of 135 respondents were included in this study. A total of 90 respondents were recruited from the AJH and 45 were recruited from the DIH. RT-qPCR was conducted on the blood samples of all respondents. Test-to-test comparability was deemed acceptable because the positive controls for each DENV, CHIKV, ZIKV, WNV, and IC consistently fell within the Ct range of 23.99-25.47, 26.06-28.13, 23.12-25.01, 23.98-29.82, and 23.95-25.38, respectively. Of the 135 respondents, 70 (51.9%) tested positive for DENV by RT-qPCR and were assigned as cases. The negative ones were assigned as controls. We also found two specimens positive for CHIKV using RT-qPCR, both of which exhibited mono-infections. No ZIKV or WNV infections were detected. WNV infection has never been reported in Indonesia previously and was not the primary focus of our study; however, it was included in the panel of the kit. We did not test for the statistical significance of the CHIKV infection features because of the limited number of cases.

A total of 70 patients who were DENV RT-qPCR–positive and 65 who were DENV RT-qPCR–negative were respectively assigned as cases and as controls, respectively, in further analysis. The number of respondents was slightly below the planned number; however, the conclusion of the rainy season led to a significant decrease in the number of hospitalized patients who fulfilled the inclusion criteria. No significant differences were observed in the demographic characteristics of the respondents who visited the two hospitals. A total of 18 and six respondents who visited AJH and DIH, respectively, reported having comorbidities, i.e. diabetes mellitus, hypertension, obesity, asthma, hyperthyroidism, hypotension, high cholesterol levels, and vertigo. Nevertheless, comorbidities did not alter the outcome of the disease because all respondents were safely discharged from the hospital. The demographic characteristics of the respondents from each hospital site and the RT-qPCR results for DENV, CHIKV, ZIKV, and WNV are shown in Table 1. Table 2 shows the demographic characteristics of the patients and controls in this study.

Clinical features of the patients infected with DENV

The average duration of the respondents' fever was 4.83 (range 2-8) days before hospital admission. Recruitment of respondents occurred when the fever was on the third day or later. Additional complaints, including headache, retro-orbital pain, myalgia, arthralgia/bone pain, skin rash, and hemorrhagic manifestations, were not statistically significant. However, when all the symptoms were combined using the WHO criteria for dengue, that is, any two other additional symptoms that accompany fever [14], it was statistically significant ($P < 0.011$). Table 3 shows the distribution of the clinical signs and symptoms among the respondents.

Laboratory characteristics of the patients infected with DENV

We used the relevant normal range of the analyzers in each hospital as the COV for blood tests. Table 4 presents the laboratory characteristics of respondents. For DENV infection, the COV for hematocrit was more than 44.4% v/v and 43% v/v to be considered hemoconcentration, for Sysmex and Mindray, respectively. The difference in hematocrit values between patients who were DENV RT-qPCR–positive and –negative was not statistically significant. COV for platelets was lower than $165 \times 10^3/\mu l$ and $150 \times 10^3/\mu l$ for Sysmex and Mindray, respectively. The mean platelets count in the DENV RT-qPCR is significantly lower ($P = 0.019$) and when analyzed as a thrombocytopenia condition ($P = 0.026$; OR 2.19, 95% confidence interval [CI] 1.06-4.52). The leukocyte count was also significantly lower in patients who were DENV RT-qPCR–positive ($P = 0.005$). The state of leukopenia was also significantly associated with DENV infection ($P = 0.017$; OR 2.28, 95% CI 1.12-4.67). AST and ALT levels were only available for 54 patients, whereas blood urea and creatinine levels were only available for 36 patients, all from AJH. Among the blood chemical tests, only the AST was significantly increased in the patients who were DENV PCR positive ($P = 0.003$, OR 5.63, 95% CI 1.75-18.10).

DENV serology test compared with DENV RT-qPCR

In total, 48 patients had been tested for the DENV serology test. A total of 12 and 39 of the 90 patients referred to the AJH were tested for DENV IgM and IgG antibodies and DENV NS-1 antigen, respectively. DENV NS-1 antigen positivity was significantly associated with DENV RT-qPCR positivity ($P < 0.001$). The distribution of DENV RT-qPCR and DENV serology for those tested is shown in Table 5. The mean Ct value and relative fluorescence units of the PCR-positive samples are detailed in Supplementary Table 1.

Discussion

The WHO issued guidelines for the diagnosis and management of DENV infection in 2011. The guidelines classify DENV infections into five categories: dengue fever and dengue hemorrhagic fever grades I-IV. The WHO criteria also include blood test values, including leukopenia, thrombocytopenia, and increased hematocrit [14]. Guidelines have also been adopted in Indonesia [5]. In our study, we found that the guidelines remained applicable to most patients infected with DENV in both hospitals, even in 2024 when this study was conducted.

Table 1
Demographic characteristics of the respondents from each hospital.

Characteristics	Atma Jaya Hospital (n = 90)	Duta Indah Hospital (n = 45)	All respondents (n = 135)
Gender			
Male	36 (40.0%)	23 (51.1%)	59 (43.7%)
Female	54 (60.0%)	22 (48.9%)	76 (56.3%)
Age (SD)	33.36 (12.58)	31.37 (9.95)	32.70 (11.77)
Body mass index (SD)	23.87 (5.40)	24.12 (5.05)	23.95 (5.27)
Day of fever before admission (SD)	4.87 (1.47)	4.76 (1.48)	4.83 (1.47)
Traveling out of town in the last week	17 (18.9%)	6 (13.3%)	23 (17.0%)
Types of dwelling			
Landed house	65 (72.2%)	34 (75.6%)	99 (73.3%)
Subsidized apartment	14 (15.6%)	10 (22.2%)	24 (17.8%)
Condominium	11 (12.2%)	1 (2.2%)	12 (8.9%)
Having comorbidity[a]	18 (13.3%)	6 (4.5%)	24 (17.8%)
Reverse transcription-quantitative polymerase chain reaction results			
DENV	45 (50.0%)	25 (55.6%)	70 (51.9%)
CHIKV	2 (2.2%)	0 (0%)	2 (1.5%)
ZIKV	0 (0%)	0 (0%)	0 (0%)
WNV	0 (0%)	0 (0%)	0 (0%)
Negative	43 (47.8%)	20 (44.4%)	63 (46.6%)

[a] Reported comorbidities included hypertension, diabetes mellitus, asthma, vertigo, hyperthyroidism, hypotension, and hypercholesterolemia.

Table 2
Characteristics of the dengue reverse transcription-quantitative polymerase chain reaction positive and negative respondents.

Characteristics	Dengue		All respondents (n = 135)
	Control (n = 65)	Case (n = 70)	
Gender			
Male	26	33	59
Female	39	37	76
Age (SD)	34.34 (13.43)	31.17 (9.84)	32.69 (11.77)
Body mass index (SD)	23.10 (5.52)	24.74 (4.95)	23.95 (5.27)
Traveling out of town in the last week	53	59	112
Types of dwelling			
Landed house	42	57	99
Subsidized apartment	16	8	24
Condominium	7	5	12
Comorbidity			
None	52	59	111
Having comorbidity[a]	13	11	24

[a] Reported comorbidities included hypertension, diabetes mellitus, asthma, vertigo, hyperthyroidism, hypotension, and hypercholesterolemia.

Table 3
Clinical symptoms of the patients infected with dengue virus.

Clinical symptoms	Dengue		Total	*P*-value[a]
	Control	Case		
Days of fever before admission	5.12 (SD 1.54)	4.66 (SD 1.39)	4.83 (SD 1.47)	0.158
Bleeding manifestation				
No bleeding	42 (31.1%)	36 (26.7%)	78 (57.8%)	0.084
Signs of bleeding	23 (17.0%)	34 (25.2%)	57 (42.2%)	
Headache				
Negative	9 (6.6%)	4 (3.0%)	13 (9.6%)	0.095
Positive	56 (41.5%)	66 (48.9%)	122 (90.4%)	
Myalgia				
Negative	17 (12.6%)	21 (15.5%)	38 (28.1%)	0.381
Positive	48 (35.6%)	49 (36.3%)	97 (71.9%)	
Arthralgia/bone pain				
Negative	22 (16.3%)	18 (13.3%)	40 (29.6%)	0.199
Positive	43 (31.9%)	52 (38.5%)	95 (70.4%)	
Retro-orbital pain				
Negative	44 (32.6%)	46 (34.1%)	90 (66.7%)	0.476
Positive	21 (15.6%)	24 (17.8%)	45 (33.3%)	
Skin rash				
Negative	41 (30.3%)	36 (26.7%)	77 (57.0%)	0.112
Positive	24 (17.8%)	34 (25.2%)	58 (43.0%)	
Fever and additional symptoms[b]				
Less than two other symptoms	10 (7.4%)	2 (1.5%)	12 (8.9%)	0.011
Two or more other symptoms	55 (40.7%)	68 (50.4%)	123 (91.1%)	

[a] Non-parametric Pearson chi-squared test was used for statistical analysis.
[b] Statistically significant.

Table 4
Laboratory features of the patients who tested positive with dengue virus polymerase chain reaction.

Laboratory parameters	Dengue virus polymerase chain reaction		Total	*P*-value	Odds ratio (95% confidence interval)
	Control	Case			
Average hemoglobin (g/dl)[a]	13.6 (SD 2.0)	14.1 (SD 1.7)	13.8 (SD 1.9)	0.162	
Average hematocrit (% v/v)[a]	39.9 (SD 5.8)	41.4 (SD 5.0)	40.7 (SD 5.4)	0.126	
Increased hematocrit	14/65 (21.5%)	23/70 (32.9%)	37/135 (27.4%)	0.135	1.67 (0.77-3.63)
Average platelet count[b] ($\times 10^3$/µl)[a]	141.6 (SD 80.3)	113.8 (SD 53.7)	127.2 (SD 69.0)	0.019	
Decreased platelet count[b]	37/65 (56.9%)	52/70 (74.3%)	89/135 (65.9%)	0.026	2.19 (1.06-4.52)
Leukocyte count[b] ($\times 10^3$/µl)[a]	5.49 (SD 3.25)	4.12 (SD 2.27)	4.77 (SD 2.86)	0.005	
Leukopenia[b]	34/65 (52.3%)	50/70 (71.4%)	84/135 (62.2%)	0.017	2.28 (1.12-4.67)
Differential blood count					
Basophils[b] (% leukocytes)[a]	0.30 (SD 0.23)	0.48 (SD 0.36)	0.39 (SD 0.31)	0.006	
Eosinophils (% leukocytes)[a]	1.11 (SD 2.13)	1.13 (SD 4.22)	1.12 (SD 3.32)	0.982	
Neutrophils (% leukocytes)[a]	64.86 (SD 14.94)	64.44 (SD 16.70)	64.66 (SD 15.75)	0.900	
Lymphocytes (% leukocytes)[a]	24.35 (SD 12.59)	23.53 (SD 14.31)	23.95 (SD 13.40)	0.773	
Monocytes (% leukocytes)[a]	9.37 (SD 4.04)	10.39 (SD 3.93)	9.87 (SD 3.99)	0.227	
Red blood cell count ($\times 10^6$/µl)[a]	4.81 (SD 0.61)	5.02 (SD 0.64)	4.92 (SD 0.63)	0.087	
Average AST[c] (U/l)[a]	44.1 (SD 38.2)	68.8 (SD 52.7)	56.9 (SD 47.5)	0.056	
Increased AST[b]	8/26 (30.8%)	20/28 (71.4%)	28/54 (51.9%)	0.003	5.63 (1.75-18.10)
Average ALT[c] (U/l)[a]	35.2 (SD 27.1)	41.8 (SD 34.2)	38.6 (SD 30.9)	0.434	
Average blood urea[c] (mg/dl)[a]	20.7 (SD 8.1)	23.2 (SD 8.8)	21.7 (SD 8.3)	0.370	
Average creatinine[c] (mg/dl)[a]	0.83 (SD 0.18)	0.92 (SD 0.24)	0.87 (SD 0.21)	0.191	

ALT, alanine transaminase; AST, aspartate transaminase.
[a] *t*-test was used for statistical analysis.
[b] Statistically significant.
[c] Laboratory test for AST (n = 54), ALT (n = 54), blood urea (n = 36), and creatinine (n = 36) were only available for Atma Jaya Hospital.

Table 5
DENV serology and polymerase chain reaction profiles of patients in Atma Jaya Hospital and Duta Indah Hospital.

Dengue serology	Dengue		Total	*P*-value[a]
	Control	Case		
DENV serology test[b]				
Not tested	47	40	87	0.048
Tested	18	30	48	
DENV IgM				
Negative	6	2	8	0.200
Positive	1	3	4	
DENV IgG				
Negative	4	2	6	0.644
Positive	3	3	6	
DENV nonstructural protein-1[b]				
Negative	10	5	15	<0.001
Positive	2	22	24	

DENV, dengue virus; Ig, immunoglobulin.
[a] Pearson chi-square was used for the statistical test.
[b] Statistically significant.

All clinical features listed as part of the diagnostic criteria in the WHO guidelines were present in patients infected with DENV. Although each of the criteria alone did not differ significantly between patients infected with DENV and those with negative results, when we used fever with at least two other signs and symptoms as the criterion, the difference was significant ($P = 0.011$). A study conducted in the Reunion Islands concluded that the WHO criteria could assist in the identification of probable dengue. The study criteria demonstrated sensitivity and specificity rates of 64% and 57%, respectively, indicating their potential utility in screening suspected cases for further testing [18]. As part of the host defense mechanisms against viral infection, the immune system relies on the cellular immune response and production of interferons and other cytokines that can initiate local inflammation and systemic responses. This leads to the flu-like symptoms that are commonly associated with viral infections. Those systemic symptoms may include fever, malaise, headache, myalgia, etc. [19].

DENV is one of the viruses that could trigger thrombocytopenic purpura and disseminated intravascular coagulation, resulting in hemorrhage [20]. Although most of the patients in this study had a mild form of DENV infection, mainly, petechiae and epistaxis, some patients could have severe forms, including gastrointestinal system bleeding. It is assumed that interactions between the host, virus, vector, and environment may play a role in this phenomenon [20]. In this study, we did not find significant differences in bleeding events between those who were DENV RT-qPCR–positive and –negative. The mild form of DENV infection observed in our study may explain this phenomenon.

Retro-orbital pain caused by DENV infections has been discussed in several studies. One hypothesis suggests that anti-DENV NS-1 antibodies may induce apoptosis in endothelial cells, including those in the blood-retinal barrier, resulting in hemorrhage and plasma leakage. Ophthalmic symptoms may manifest in the anterior and posterior segments and are associated with thrombocytopenia [21]. In our study, we found that the occurrence of retro-orbital pain was nearly equal in respondents who tested positive and negative for DENV by RT-qPCR.

Hematologic tests are an important modality for physicians to diagnose DENV infections. The WHO guidelines include findings of leukopenia, thrombocytopenia, and increasing hematocrit levels [14]. A study using mouse models concluded that thrombocytopenia is caused by a reduction in megakaryocytes [22]. A recent study of infected interferon-α/β-γ-receptor double-knockout mice with recombinant chimeric *Fla-*

vivirus found that general bone marrow suppression occurred in the models, and high viremic levels were detected in bone marrow [23]. This may clarify the significance of leukopenia and thrombocytopenia in the patients who are DENV-positive in our study.

An increase in hematocrit levels is part of the WHO guidelines criteria [14]. We did not observe a significant difference in hematocrit levels between patients who were DENV-positive and -negative. The indicator in the criteria is an increase in the hematocrit value, which can only be determined if the baseline value is available. Several factors may have influenced this result. Anemia occurs frequently in developing countries and presents as a lower hemoglobin level, which, in turn, affects the initial hematocrit value; therefore, the hematocrit level without the baseline value is difficult to interpret. One way to address this issue is to conduct serial hematocrit tests over the course of the disease. However, it is important to note that this approach increases the cost of dengue treatment and is challenging for dengue fever cases that do not require hospitalization.

Our study involved the use of different blood analyzers in hospitals, that is, Sysmex and Mindray. Different analyzers have different reference values. To address this issue, blood tests, such as elevation or decrease in hematocrit, leukocyte levels, and platelets, were categorized by referencing the standard values provided by each analyzer. However, the interpretation of the mean value was affected by different analyzers.

Liver injury caused by DENV infection has been reported in several studies [24,25]. Injury can be caused by a direct cytopathic effect, immune-mediated injury, or poor hepatic perfusion [26]. In our study, we found a significant increase in serum AST levels but not in serum ALT levels. The upper limit of the normal values (38 U/l) was used as the COV and concluded that even in the patients who were DENV-negative, the mean AST level is already slightly higher (44.1 U/l), although the mean AST level of the patients who were DENV-positive is much higher at 68.8 U/l. This finding suggests that the DENV infection in our respondents could cause liver injury. However, in Indonesia, patients with fever frequently use acetaminophen as an antipyretic. High doses of acetaminophen can increase serum transaminase levels. In this study, all acetaminophen dosages used were below the maximal dosage; however, we could not rule out their use before admission to the hospital. We did not observe any significant increase in serum ALT levels in our respondents. A study by Wang et al. [25] and by Prommalikit et al. [27] reported that the prevalence of increased ALT was approximately half that of AST. The number of respondents in our study could accommodate the differences in AST levels but might not have statistical power for differences in ALT levels.

DENV serology tests were performed on some respondents before they were admitted to the AJH. Of these, 12 respondents had been tested for a combination of DENV IgM and IgG and 39 were tested for the DENV NS-1 antigen. Regarding the analysis, the DENV NS-1 antigen test is a reliable predictor for DENV infection but not for DENV IgG or IgM. The DENV NS-1 antigen directly detects components of the virus, indicating the presence of the virus in the serum, whereas antibodies may only be positive in the later course of the disease. Our study identified two cases in which the DENV NS-1 antigen was detected but the DENV RT-qPCR was negative. Antigen tests were conducted before hospitalization, whereas DENV RT-qPCR was conducted on blood samples within the first few days of hospitalization. Different time points may account for the discrepancies observed in these findings. The limited number of tested DENV antibodies prevents definitive conclusions regarding their association with DENV infection. However, one can argue that DENV IgM positivity is also a good test for detecting DENV infection. For practical reasons, the DENV NS-1 antigen test is preferred over the DENV antibody test.

In this study, we identified two cases of CHIKV infection. The sample size was limited; therefore, we could not perform any statistical analysis. This confirms that CHIKV continues to circulate throughout Indonesia. A systematic review of CHIKV infections in 2019 concluded that the incidence of chikungunya in Indonesia ranged between 0.16 and 36.2 cases per 100,000 person-years [28]. Research by Arif et al. [7] reported an incidence of 3.7% of acute CHIKV infections. In our study, the incidence was two per 135 (1.5%); both patients had mono-infections. A similar situation was reported in a previous published study [7]. Although the incidence of CHIKV infection is considerably low, the same vector is present and capable of transmitting the virus, similar to DENV. Differentiating between CHIKV and DENV infections is important because the administration of non-steroidal anti-inflammatory drugs as analgesics should successfully rule out DENV infection [29]. In addition, neither ZIKV nor WNV infections were detected in our study. Nevertheless, clinicians, civilians, and the government must exercise caution regarding the potential spread of ZIKV in Indonesia, especially given the virus' circulation history in Singapore, a country that frequently travels with Jakarta [10].

In this study, 63 patients with acute febrile illnesses other than DENV and CHIKV infections were identified. Common bacterial infections in the patients who were PCR-negative had been ruled out because it was part of the exclusion criteria in this study. Several differential diagnoses of acute febrile illnesses should be considered. Typhoid fever is still considered endemic in Indonesia [30] but was ruled out in this study. Leptospirosis is an infection that occurs after flooding during the rainy season [31]. A study in Bandung also listed murine typhus, caused by *Rickettsia typhi*, as a possibility [32]. We ruled out leptospirosis and murine typhus in our study because the different subsequent courses of the diseases in our respondents presented different clinical signs and symptoms than those of the aforementioned diseases.

All patients presented in the current study had mild forms of dengue. Several studies have concluded that certain serotypes of dengue, particularly, serotype-2 and -3, are associated with severe disease manifestations [33,34]. In this study, we did not assess the serotype of the DENV. Some patients in the present study underwent serologic tests. In these tests, dengue IgG was positive. The limited availability of IgG for dengue in our data may be significant because multiple studies suggest that secondary and concomitant infections with several serotypes could lead to more severe manifestations of the disease [33,35,36]. Studies of dengue serotypes and secondary or concomitant serotype infections in Jakarta are of significant interest.

Strengths and limitations of the study

To the best of our knowledge, this is the first post–COVID-19 study on dengue from Indonesia to use RT-qPCR for infection confirmation. One report indicated that COVID-19 vaccines, particularly, the adenovirus vector vaccine, could induce thrombotic thrombocytopenia, potentially influencing the progression of DENV infections [37]. Our study provides insights into the current status of DENV infections in Indonesia.

Nevertheless, our study has some limitations. The number of respondents in the control group was smaller than the expected minimum sample size. This may account for the lack of significance in certain analyses, where only those exhibiting stronger associations achieved statistical significance. The different blood analyzers used in hospitals (Sysmex and Mindray) may yield divergent results despite using the respective reference values for each analyzer to categorize the test results. The interpretation of blood test results would have been facilitated if both hospitals had used identical analyzers. The lack of blood chemical tests for DIH and the limited number of respondents hindered the analysis of ALT and DENV serologic tests, potentially affecting the analysis if complete data were available. Most respondents in our study had a mild form of dengue infection; therefore, we could not analyze the clinical features of the severe form of dengue infection.

Conclusion

This study concluded that the WHO criteria for the signs and symptoms of DENV infection, particularly, in the mild form, remain applicable in Indonesia. Similarly, our study confirmed that leukopenia and

thrombocytopenia are the criteria for DENV infection. Although a one–time point hematocrit test offers limited predictive value, subsequent testing could increase its predictive value for DENV infections. The WHO criteria are applicable to most hospitals in Indonesia. This should serve as a primary screening method for patients with suspected dengue.

The identification of CHIKV infections in this study highlights its prevalence in Indonesia. In cases of doubt, additional tests are recommended to confirm the presence of CHIKV infection. Despite the absence of ZIKV and WNV infections in the current investigation, it remains essential to implement preventive measures against the transmission of both viruses, considering that the vector is present in Indonesia.

Declarations of competing interest

The authors have no competing interests to declare.

Funding

The authors received an internal institutional research grant from a home-based university. The GeneCraft Laboratory provided the RNA extraction kits used for this study. None of the funders had a role in the study design, data analysis, data interpretation, writing of the report, decision to submit for publication, or any aspect pertinent to the study.

Ethical approval

The study protocol was reviewed by the Internal Research Ethics Committee and granted clearance no. 21/05/KEP-FKIKUAJ/2023. The inclusion of an additional hospital in the study was requested as an amendment and was granted clearance no. 11/02/KEP-FKIKUAJ/2024.

Acknowledgment

The authors would like to thank all the doctors from the Department of Internal Medicine, AJH for the recruitment of respondents, and laboratory analysts from AJH for their help during the hematologic profile and blood sample collection: Dr. Firmansye Ika Panggulu, MARS, Dyah Rita Wulangun, S. Kep., Ns., MPH, Ns. Frakassona Karisiane, S. Kep, Nengah Mariani, Amd Kep, Kayunah, Amd Ak, and Ns. The authors thank Helita Kikisan Sandra and S. Kep from DIH for their contributions to this study. The authors would also like to thank David Kristin, S. Si, and Felicia Anggraini, S. Biomed for their help during the laboratory work.

Author contributions

SA—conceptualization, methodology, funding acquisition, laboratory method optimization, data collection, data management, data analysis, supervision, writing the first draft, and reviewing the manuscript; MMMK—Conceptualization, methodology, funding acquisition, laboratory method optimization, data analysis, supervision, writing the first draft, reviewing the manuscript; AH—methodology, respondent recruitment, laboratory work, data collection, data management, data analysis, reviewing the manuscript; AIT—respondent recruitment, laboratory work, data collection, reviewing the manuscript; AMJ—laboratory work, data collection, reviewing the manuscript; FC—conceptualization, methodology, respondent recruitment, data collection, data analysis, reviewing the manuscript; LL—conceptualization, methodology, respondent recruitment, data collection, data analysis, reviewing the manuscript; JWW—conceptualization, methodology, laboratory work, data collection, data analysis, reviewing the manuscript.

Data sharing statement

All data relevant to the study have been included in the article or uploaded as supplementary information.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2025.100612.

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