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Research

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Correlation of polymerase chain reaction results with hematocrit levels and platelet counts in dengue patients in Batam City

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Abstract

Background Dengue hemorrhagic fever (DHF) is a viral disease transmitted by *Aedes aegypti* mosquitoes, posing global public health challenge. The Riau Islands Province has the highest incidence of DHF in Indonesia.

Objective This study aimed to investigate the relationship between hematocrit and platelet levels with the cycle threshold (Ct) values of polymerase chain reaction (PCR) results in DHF cases in Batam City, Riau Islands Province.

Methods A descriptive correlation study was conducted using data from 102 patients infected with the dengue virus. Hematocrit and platelet counts were measured using a hematology analyzer, while Ct values for DENV1, DENV2, DENV3, and DENV4 were obtained through real-time qRT-PCR. Pearson's correlation test was employed to analyze the relationship between these variables.

Results The study found no significant gender difference in DHF incidence (males: 50%, females: 50%). The highest prevalence was observed in the 6–11 years age group (44.1%), followed by the 12–18 years group (25.5%), the >18 years group (24.5%), and the 1–5 years group (11.8%). DENV3 was identified as the dominant serotype. No statistically significant correlation was found between Ct values and hematocrit (p = 0.607) or platelet counts (p = 0.323).

Conclusion DHF cases in this study showed no gender disparity, with the most affected group being children aged 6–11 years, and DENV3 was the prevalent serotype. Ct values did not show a statistically significant correlation with hematocrit levels or platelet counts, suggesting that these hematological parameters may not predict viral load in DHF cases.

Keywords: cycle threshold (Ct) value | dengue virus serotype | hematocrit level | platelet count | qRT-PCR

Introduction

In 2020, Indonesia recorded 108,303 cases of dengue fever (DF). The following year, the number of cases decreased to 70,928, with a total of 689 deaths reported across 467 districts/cities. The Riau Islands Province had the highest incidence rate in 2021, with 80.9 cases per 100,000 population, East Kalimantan with 78.1, and Bali with 59.8 cases (Schaefer *et al.*, 2017).

Dengue fever is caused by the dengue virus, a member of the Flavivirus family with single-stranded RNA, comprising four distinct serotypes (DENV1–4). The virus is transmitted by female mosquitoes of *Aedes aegypti* and *Aedes albopictus*, both prevalent in tropical and subtropical regions. In Indonesia, dengue fever has become endemic in many areas, with cases increasing annually (Ministry of Health, 2021). Although the four virus serotypes are antigenically similar, they do not

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provide cross-protection after infection. Infection by one serotype induces long-term immunity only to that specific serotype, leaving individuals susceptible to the others (Reich *et al.*, 2013).

The clinical manifestations of dengue virus infection range from asymptomatic cases and mild, nonspecific febrile illness to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Severe forms of DHF, when accompanied by complications, can lead to death (Andriani *et al.*, 2018; WHO, 2011). A complete blood count (CBC) is crucial in diagnosing dengue fever.

Platelets play a vital role in preventing bleeding by forming blood clots. The average platelet count in humans ranges from 150,000 to 400,000 cells per microliter of blood, while the hematocrit value is approximately 40% to 48% in men and 37% to 43% in women (Gandasoebrata, 2010). Monitoring platelet counts helps assess the severity of thrombocytopenia in DHF patients (Ojha *et al.*, 2017). An increased hematocrit value, known as hemoconcentration, or a decrease in hematocrit by more than 20% following fluid therapy, indicates increased vascular permeability (WHO, 2011). DHF causes increased capillary permeability, reduced plasma blood volume, and increased hemostasis disorders, including vasculopathy, thrombocytopenia, and coagulopathy (WHO, 2009). Therefore, measuring hematocrit and platelet counts is essential for diagnosing and managing DHF (WHO, 2009).

Several studies have investigated the correlation between hematocrit and platelet counts, but the results have been varied. Hukom *et al.* (2013) found no significant correlation between hematocrit and platelet counts in dengue fever patients. Conversely, Livina *et al.* (2014) reported a weak negative correlation between hematocrit and platelet counts. This study further examined the correlation between these values in Batam City patients infected with the dengue virus.

Dengue serotyping can be performed using real-time polymerase chain reaction (RT-PCR) (Andriyoko *et al.*, 2012; Yung *et al.*, 2015). RT-PCR testing is recommended for DHF patients in Batam City to identify the specific viral serotype. Quantitative PCR with reverse transcriptase (qRT-PCR) is the most accurate and sensitive diagnostic standard. However this testing is generally available only in major hospitals and central health laboratories in provincial capitals.

This study assessed the correlation between hematocrit and platelet counts with the cycle threshold (Ct) values obtained from serotype-specific polymerase chain reaction testing in DHF patients in Batam City. The results of this study were expected to provide valuable insights into the utility of hematocrit and platelet counts in supporting the diagnosis of DHF and guiding appropriate therapeutic interventions.

Methods

Study period and location

Sample collection was conducted from September to November 2023. RT-qPCR analyses were carried out at the Laboratory of the Batam Class I Health and Disease Control Center (BTKLPP), and hematological examinations were conducted at Batu Aji Public Health Center, Batam City, Riau Islands Province.

Data collection

This study was approved by the Health Research Ethics Committee of Politeknik Kesehatan Kemenkes Bandung with ethical clearance number No.58/KEPK/EC/XII/23. A descriptive correlational method was employed to analyze the relationship between dengue virus serotypes and hematocrit and platelet values. Blood samples from DHF patients at Batu Aji Public Health Center were sent to the BTKLPP Class I Laboratory in Batam for qRT-PCR analysis. At the same time, hematological assessments were conducted at the health center. Primary samples were collected from patients who visited the Batu Aji Public Health Center between September and November 2023, forming the study population. Primary data were gathered from this period, while secondary data were sourced from the previous three years (2021, 2022, and 2023).

Inclusion and exclusion criteria

Inclusion and exclusion criteria were applied to determine patient eligibility. Inclusion criteria were as follows: DHF patients with an illness duration of 2–7 days from fever onset, complete medical records, comprehensive blood tests including platelet count, leukocyte count, hematocrit value, hemoglobin level, and positive PCR results. Exclusion criteria included incomplete medical records, platelet transfusion, a history of medications that may suppress bone marrow function, a history of hematological disorders, and a non-sigmoid Ct value graph after repeat testing. Of the 123 samples examined, 102 met the inclusion criteria, while 21 were excluded due to incomplete platelet or hematocrit data or negative qRT-PCR results (**Table 1**).

Table 1 Inclusion and exclusion criteria for study samples

Year	Total samples	Inclusion	Exclusion	Remarks
2021	50	43	7	Incomplete hematocrit or platelet data
2022	55	50	5	Incomplete hematocrit or platelet data
2023	18	9	9	Incomplete hematocrit or platelet data; negative PCR results
Total	123	102	21	102 samples met inclusion criteria

Hematocrit and platelet examination

Hematocrit and platelet counts were measured from blood samples collected in tubes containing tri potassium ethylenediaminetetraacetic acid (K3EDTA). The blood samples were analyzed using an automated hematology analyzer (Samsung LABGEO HC10 IVD-C10A/EU, Samsung Electronics Co., Ltd., South Korea).

Viral RNA extraction and qRT-PCR assay

Viral RNA was extracted using the ExgeneTM Viral DNA/RNA kit, and the dengue virus serotype was determined using the VIASURE Dengue Serotyping Real-Time PCR Detection Kit (VS-DES106L). The procedure was performed according to the manufacturer's protocol (VIASURE Dengue Serotyping SOP). The qRT-PCR was conducted on a Thermal Cycler PCR machine (Biorad CFX96, Bio-Rad Laboratories Inc., California, USA). The protocol included one cycle of reverse transcription at 45°C for 15 minutes, initial denaturation at 95°C for 2 minutes, followed by 45 cycles of denaturation at 95°C for 10 seconds and extension at 60°C for 50 seconds. All procedures were repeated three times.

Data Analysis

Data analysis was conducted descriptively to determine the proportion of patients by gender, age, and virus serotype. Hematocrit values, platelet counts, and Ct values from qRT-PCR results were analyzed to obtain the minimum, maximum, and average values, which were then used to determine the frequency distribution of each dengue virus serotype. The data followed a normal distribution. Pearson correlation tests were performed using SPSS version 25 to assess the relationship between Ct values from qRT-PCR results and hematocrit and platelet counts.

Results

The study results indicate an equal proportion of male and female participants. The age group most affected by dengue was 6–11 years old. Among the dengue virus serotypes identified, DENV3 was the most prevalent, followed by DENV1 (**Figure 1**).

The hematocrit levels, platelet counts, and Ct values for the dengue virus serotypes, stratified by gender, are presented in **Table 2**. Among males, the average hematocrit value was 44.60%, the platelet count was 117.20×10^3 cells/µL, and the Ct value was 28.07. In females, the average hematocrit value was 41.70%, the platelet count was 141.02×10^3 cells/µL, and the Ct value was 27.27.

The frequency analysis of hematocrit levels, platelet counts, and Ct values across different age groups of DHF patients revealed no significant differences among the age groups (**Table 3**). The hematocrit values remained within the normal range (37%–43% for women and 40%–48% for men), while all platelet counts were below the normal range (150,000–400,000 cells per microliter of blood) (Gandasoebrata, 2010).

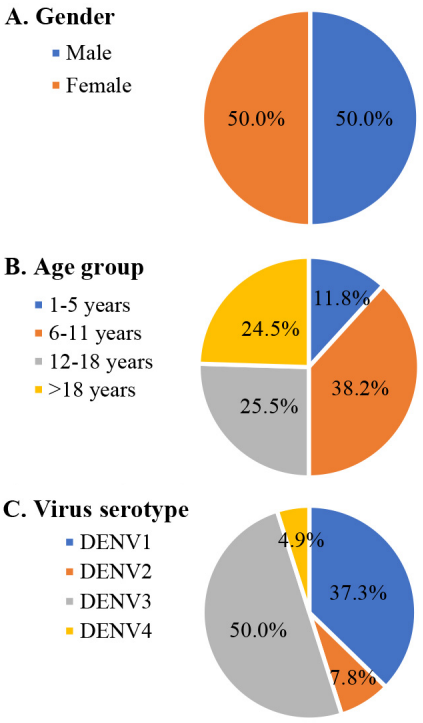

Figure 1 Percentage of dengue hemorrhagic fever (DHF) cases based on gender (A), age group (B), and virus serotype (C) from a total of 102 study samples.

Table 2 Hematocrit, platelet counts, and Ct values by gender in dengue hemorrhagic fever (DHF) patients

Gender	N	Hematocrit (%) (mean ± stdev)	Platelet count ($\times10^3$ cells/µL) (mean ± stdev)	Ct value (mean ± stdev)
Male	51	44.60 ± 5.00[a]	117.20 ± 52.74[a]	28.07 ± 4.69[a]
Female	51	41.70 ± 4.42[a]	141.02 ± 49.20[a]	27.27 ± 4.82[a]

The same superscript letters within the same column indicate no significant difference ($p > 0.05$).

Table 3 Hematocrit, platelet counts, and Ct values by age group in dengue hemorrhagic fever (DHF) patients

Age group	N	Hematocrit (%) (mean ± stdev)	Platelet count ($\times10^3$ cells/µL) (mean ± stdev)	Ct value (mean ± stdev)
1–5 years	12	40.07 ± 3.78[a]	122.58 ± 43.28[a]	28.32 ± 5.68[a]
6–11 years	39	41.82 ± 3.92[a]	142.90 ± 59.65[a]	27.64 ± 4.92[a]
12–18 years	26	44.60 ± 5.48[a]	112.69 ± 42.59[a]	27.49 ± 4.70[a]
>18 years	25	44.71 ± 4.99[a]	130.83 ± 49.36[a]	27.59 ± 4.24[a]

The same superscript letters within the same column indicate no significant difference ($p > 0.05$).

The qRT-PCR results from 102 specimens revealed that DENV3 was the most common serotype, found in 51 cases. The average hematocrit value in DENV3 cases was 43.38%, with a platelet count of 126.27×10^3 cells/µL and a Ct value of 28.09. DENV1 followed in prevalence, while DENV2 and DENV4 were the least common serotypes (**Table 4**).

Pearson correlation analysis between Ct values and hematocrit and platelet counts is summarized in **Table 5**. The correlation test yielded $p>0.05$, indicating no significant correlation between the variables under study and the Ct values.

Discussion

The findings of this study indicate no significant difference in the number of DHF (Dengue Hemorrhagic Fever) patients based on gender (**Figure 1**). This result contrasts with Harmawan's (2017) study, which reported a majority of DHF patients were female (38/69, 55.1%), and the study by Birman *et al.* (2023), which found that the majority of DHF patients were male (52/97, 53.6%). These discrepancies may be attributed to factors such as activity levels and exposure to mosquito bites. Males over 15 years are more susceptible to infection due to their higher likelihood of engaging in outdoor activities, which increases the risk of exposure to dengue virus-carrying mosquitoes. Other studies have shown that the risk of DHF infection is nearly equal between males and females (Umaya *et al.*, 2013; Hariawati *et al.*, 2022).

The age distribution of cases showed that the 6–11 years age group had the highest incidence of dengue virus infection, followed by the 12–18 years age group and the >18 years age group. This finding is consistent with the study by Dias Júnior *et al.* (2017) in São Luís, Maranhão, Brazil, which indicated that children under 15 years old are the most vulnerable group, and with Pangestika *et al.* (2022) in Bali, which identified children aged 5–9 years as the most frequent dengue patients at Sanglah General Hospital.

Children under 12 years old are more likely to engage in outdoor activities, such as playing or attending school, which increases their risk of being bitten by Aedes aegypti mosquitoes that are active during the day (WHO, 2012). Children aged 5–15 years have an immature immune system, making them more susceptible to DHF (Yati & Nababan, 2017). In this study, DENV3 was the predominant serotype, consistent with previous research (Utama *et al.*, 2019; Sasmono *et al.*, 2019; Nainggolan *et al.*, 2023) that also reported DENV3 as the dominant serotype. Annual surveys in various regions of Indonesia have also shown the dominance of the DENV3 serotype. Meanwhile, DENV4 was less prevalent, likely due to DENV4 being more commonly transmitted by Aedes albopictus, a secondary vector.

The hematocrit levels of DHF patients in this study did not differ from normal hematocrit values, while the platelet counts were lower than normal, confirming the diagnosis of DHF. The variation in patient characteristics in this study may account for these differences. The correlation analysis between Ct values, hematocrit levels, and platelet counts revealed no significant relationships. Several factors, such as variations in hematocrit levels, platelet counts, or dengue virus infection factors, may have influenced these results. Two main theories related to dengue virus infection are secondary infection and antibody-dependent enhancement (ADE). Secondary infection by the dengue virus can lead to more severe symptoms. Antibodies generated during the initial infection may bind to the new dengue virus, causing an excessive immune response (ADE hypothesis). The binding of the virus to non-neutralizing antibodies can activate macrophages and increase viral replication within macrophages (Sun *et al.*, 2019; Narayan & Tripathi, 2020).

Dengue virus infection begins when the vector feeds on the host's blood, transmitting the virus into the body. The immune response to dengue virus infection involves the humoral immune response, which includes the production of antibodies that neutralize the virus, complement-mediated osmotic lysis, and antibody-mediated cytotoxicity involving T-helper cells (CD4) and cytotoxic T cells (CD8) (Silveira *et al.*, 2018). Dengue virus infects macrophages, monocytes, and dendritic cells through the endocytosis process mediated by the binding of the antibody-virus complex to the Fc receptor (Bournazos *et al.*, 2020), activating T-helper cells and cytotoxic T cells. This process produces two

Table 4 Hematocrit, platelet counts, and Ct values by dengue virus (DENV) serotype

Virus serotype	N	Hematocrit (%) (mean ± stdev)	Platelet count ($\times10^3$ cells/µL) (mean ± stdev)	Ct value (mean ± stdev)
DENV1	38	43.28 ± 4.40 [a]	139.63 ± 46.49 [a]	27.38 ± 4.90 [a]
DENV2	8	42.01 ± 6.73 [a]	96.75 ± 31.44 [a]	26.05 ± 7.03 [a]
DENV3	51	43.38 ± 5.05 [a]	126.27 ± 53.36 [a]	28.09 ± 4.28 [a]
DENV4	5	41.60 ± 5.26 [a]	153.60 ± 62.95 [a]	28.19 ± 4.82 [a]

The same superscript letters within the same column indicate no significant difference ($p > 0.05$).

Table 5 Pearson correlation between Ct values and hematocrit/platelet counts

Data group	df	Pearson correlation	Sig. value	Conclusion
Hematokrit	102	0.099	0.323 (>0.05)	No correlation
Trombosit	102	-0.052	0.607 (>0.05)	No correlation

proteins essential to the immune system: lymphokines and interferon-γ. Interferon-γ activates macrophages to combat the infection (Keeler & Fox, 2021). Macrophage activation leads to the secretion of inflammatory mediators such as TNFα, IL-1, PAF, IL-6, and histamine. In DHF, endothelial cells experience dysfunction, causing plasma leakage from damaged blood vessels (Puc *et al.*, 2021).

Most DHF patients experience thrombocytopenia, a condition in which the platelet count in the bloodstream is lower than in daily condition. The study results showed a decrease in platelet counts. The causes of thrombocytopenia in DHF patients include megakaryocyte infection, reduced platelet production in the bone marrow, increased destruction and clearance of platelets from peripheral blood, or antibody reactions with platelets and endothelial cells (Chao *et al.*, 2019).

DHF can cause symptoms ranging from mild to severe. The severity of the disease is influenced by several factors, such as viral load, age, gender, and health status (Vogels *et al.*, 2019). This study has limitations because it did not measure the severity of the disease in patients. Identification was based only on age, gender, and serotype, while viral load and patient health status were not analyzed.

Conclusion

This study demonstrates that the number of DHF cases does not differ between genders, with the 6–11 years age group being the most affected and DENV3 being the dominant virus serotype. The correlation analysis indicates no significant relationship between Ct values and hematocrit levels or platelet counts in DHF patients.

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Conflict of interest All authors declare no conflicts of interest in this study.

Author contributions KS conducted the research and drafted the manuscript; FM and EH assisted in analyzing the research results; BN designed the research methodology and provided input throughout the research process.

References

Andriani S, Aryati A, Hadi U. 2018. Correlation of dengue virus serotype and DVI severity in adult patients. *Indonesian Journal of Clinical Pathology and Medical Laboratory*, 24(2): 185–190. DOI: 10.24293/ijcpml.v24i2.1322.

Andriyoko B, Parwati I, Tjandrawati A, Lismayanti L. 2012. Penentuan serotipe virus dengue dan gambaran manifestasi klinis serta hematologi rutin pada infeksi virus dengue. *Majalah Kedokteran Bandung*, 44(4): 253–260. DOI: 10.15395/mkb.v44n4.138.

Birman Y, Setiawan P, Hansah RB. 2023. Profil demam berdarah dengue di RSUP Dr M. Djamil Padang tahun 2020-2022. *Nusantara Hasana Journal*, 2(8): 42–54.

Bournazos S, Gupta A, Ravetch JV. 2020. The role of IgG Fc receptors in antibody-dependent enhancement. *Nature Reviews. Immunology*, 20(10): 633–643. DOI: 10.1038/s41577-020-00410-0.

Chao CH, Wu WC, Lai YC, Tsai PJ, Perng GC, Lin YS, Yeh TM. 2019. Dengue virus nonstructural protein 1 activates platelets via Toll-like receptor 4, leading to thrombocytopenia and hemorrhage. *PLoS Pathogens*, 15(4): e1007625. DOI: 10.1371/journal.ppat.1007625.

Dias Júnior JJ, Branco MDRFC, Queiroz RCS, Santos AMD, Moreira EPB, Silva MDSD. 2017. Analysis of dengue cases according to clinical severity, São Luís, Maranhão, Brazil. *Revista do Instituto de Medicina Tropical de São Paulo*, 59: e71. DOI: 10.1590/S1678-9946201759071.

Gandasoebrata R. 2010. Penuntun laboratorium klinik. Cetakan ke-16. Jakarta (ID): Dian Rakyat.

Hariawati, Tosepu R, Effendy DS. 2022. Dengue hemorrhagic fever cases by gender in the North Buton Regency in the 2018-2020 period. *3rd International Conference on Advance & Scientific Innovation ICASI–Life Sciences Chapter, KnE Life Sciences,* Pp 148–153. DOI: 10.18502/kls.v0i0.11791.

Harmawan D. 2017. Hubungan karakteristik klien demam berdarah dengue (DBD) dengan kejadian demam berdarah dengue (DBD) di wilayah kerja Puskesmas I Purwokerto Timur Kabupaten Banyumas. [Disertasi Doktor]. Purwokerto (ID): Universitas Muhammadiyah.

Hukom AO, Warouw SM, Memah M, Mongan AE. 2013. Hubungan nilai hematokrit dan nilai jumlah trombosit pada pasien demam berdarah dengue. *Jurnal e-Biomedik*, 1(1): 707–711. DOI: 10.35790/ebm.v1i1.4154.

Keeler SP, Fox JM. 2021. Requirement of Fc-Fc gamma receptor interaction for antibody-based protection against emerging virus infections. *Viruses*, 13(6): 1037. DOI: 10.3390/v13061037.

Kemenkes [Kementerian Kesehatan Republik Indonesia]. 2021. Profil kesehatan Indonesia tahun 2020. Hardhana B, Sibuea F, Widiantini W (eds). Jakarta (ID): Kementerian Kesehatan Republik Indonesia.

Livina A, Rotty LW, Panda L. 2014. Hubungan trombositopenia dan hematokrit dengan manifestasi perdarahan pada penderita demam dengue dan demam berdarah dengue. *e-CliniC*, 2(1).

Nainggolan L, Dewi BE, Hakiki A, Pranata AJ, Sudiro TM, Martina B, van Gorp E. 2023. Association of viral kinetics, infection history, NS1 protein with plasma leakage among Indonesian dengue infected patients. *PloS One*, 18(5): e0285087. DOI: 10.1371/journal.pone.0285087.

Narayan R, Tripathi S. 2020 Intrinsic ADE: the dark side of antibody dependent enhancement during dengue infection. *Frontiers in Cellular and Infection Microbiology,* 10: 580096. DOI: 10.3389/fcimb.2020.580096.

Ojha A, Nandi D, Batra H, Singhal R, Annarapu GK, Bhattacharyya S, Seth T, Dar L, Medigeshi GR, Vrati S, Vikram NK, Guchhait P. 2017. Platelet activation determines the severity of thrombocytopenia in dengue infection. *Scientific Reports*, 7: 41697. DOI: 10.1038/srep41697.

Pangestika NPW, Gustawan IW, Utama IMGDL. 2022. Karakteristik anak dengan infeksi dengue di RSUP Sanglah, Denpasar, Bali. *Intisari Sains Medis*, 13(1): 232–237. DOI: 10.15562/ism.v13i1.1261.

Puc I, Ho TC, Yen KL, Vats A, Tsai JJ, Chen PL, Chien YW, Lo YC, Perng GC. 2021. Cytokine signature of dengue patients at different severity of the disease. *International Journal of Molecular Sciences,* 22(6): 2879. DOI: 10.3390/ijms22062879.

Reich NG, Shrestha S, King AA, Rohani P, Lessler J, Kalayanarooj S, Yoon IK, Gibbons RV, Burke DS, Cummings DA. 2013. Interactions between serotypes of dengue highlight epidemiological impact of cross-immunity. *Journal of the Royal Society, Interface*, 10(86): 20130414. DOI: 10.1098/rsif.2013.0414.

Sasmono RT, Kalalo LP, Trismiasih S, Denis D, Yohan B, Hayati RF, Haryanto S. 2019. Multiple introductions of dengue virus strains contribute to dengue outbreaks in East Kalimantan, Indonesia, in 2015-2016. *Virology Journal*, 16(1): 93. DOI: 10.1186/s12985-019-1202-0.

Schaefer TJ, Panda PK, Wolford RW. 2017. Dengue fever. *In*: StatPearls. Treasure Island (FL): StatPearls Publishing.

Silveira GF, Wowk PF, Cataneo AHD, Dos Santos PF, Delgobo M, Stimamiglio MA, Lo Sarzi M, Thomazelli APFS, Conchon-Costa I, Pavanelli WR, Antonelli LRV, Báfica A, Mansur DS, Dos Santos CND, Bordignon J. 2018. Human T lymphocytes are permissive for dengue virus replication. *Journal of Virology,* 92(10): e02181-17. DOI: 10.1128/JVI.02181-17.

Sun P, Williams M, Nagabhushana N, Jani V, Defang G, Morrison BJ. 2019. NK cells activated through antibody-dependent cell cytotoxicity and armed with degranulation/IFN-γ production suppress antibody-dependent enhancement of dengue viral infection. *Scientific Reports*, 9(1): 1109. DOI: 10.1038/s41598-018-36972-2.

Umaya R, Faisya AF, Sunarsih E. 2013. Hubungan karakteristik pejamu, lingkungan fisik dan pelayanan kesehatan dengan kejadian demam berdarah dengue (DBD) di wilayah kerja Puskesmas Talang Ubi Pendopo tahun 2012. *Jurnal Ilmu Kesehatan Masyarakat*, 3(4): 262–269.

Utama IMS, Lukman N, Sukmawati DD, Alisjahbana B, Alam A, Murniati D, Utama IMGDL, Puspitasari D, Kosasih H, Laksono I, Karyana M, Karyanti MR, Hapsari MMDEAH, Meutia N, Liang CJ, Wulan WN, Lau CY, Parwati KTM. 2019. Dengue viral infection in Indonesia: epidemiology, diagnostic challenges, and mutations from an observational cohort study. *PLoS Neglected Tropical Diseases*, 13(10): e0007785. DOI: 10.1371/journal.pntd.0007785.

Vogels CBF, Rückert C, Cavany SM, Perkins TA, Ebel GD, Grubaugh ND. 2019. Arbovirus coinfection and co-transmission: A neglected public health concern? *PLoS Biology,* 17(1): e3000130. DOI: 10.1371/journal.pbio.3000130.

WHO [World Health Organization]. 2009. Dengue: guidelines for diagnosis, treatment, prevention and control. Geneva (CH): World Health Organization.

WHO [World Health Organization]. 2011. Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever. *SEARO Technical Publication Series No. 60*. World Health Organization, Regional Office for South-East Asia.

WHO [World Health Organization]. 2012. Global strategy for dengue prevention and control 2012–2020. Geneva (CH): World Health Organization.

Yati AW, Nababan RM. 2017. Hubungan kadar kolesterol total dan kadar albumin dengan kebocoran plasma pada demam berdarah dengue. *Jurnal Majority*, *6*(3): 148–152.

Yung CF, Lee KS, Thein TL, Tan LK, Gan VC, Wong JGX, Lye DC, Ng LC, Leo YS. 2015. Dengue serotype-specific differences in clinical manifestation, laboratory parameters and risk of severe disease in adults, Singapore. *The American Journal of Tropical Medicine and Hygiene*, 92(5): 999–1005. DOI: 10.4269/ajtmh.14-0628.