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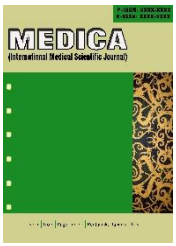

## Impact of Sample Delay on Leukocyte and Platelet Parameters in Dengue Fever Patients: A Hematological Analysis


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**Abstrak**

The diagnosis of Dengue Fever (DF) involves physical examination, laboratory tests, and confirmation primarily through thrombocytopenia and leukocyte abnormalities. Laboratory procedures play a crucial role; however, delays in complete blood count (CBC) analysis may influence hematological parameters, potentially affecting diagnostic accuracy. The research aims to determine the effect of 2-hour and 4-hour delays in CBC testing on leukocyte and platelet counts in patients with Dengue Fever. This experimental study used a posttest-only control group design with purposive sampling. Blood samples from DF patients were divided into three groups: immediate testing, 2-hour delay, and 4-hour delay. Data were analyzed using the Shapiro-Wilk test followed by Multivariate Analysis of Variance (MANOVA). The results show that the mean leukocyte and platelet counts in the immediate testing group were $4.86\times10^3$/µL and $61.55\times10^3$/µL, respectively. In the 2-hour delay group, leukocyte counts slightly decreased to $4.79\times10^3$/µL and platelet counts increased to $65.77\times10^3$/µL. In the 4-hour delay group, leukocyte counts increased to $4.85\times10^3$/µL while platelet counts decreased to $64\times10^3$/µL. MANOVA results (Sig. = 1.000) indicated no significant differences among the groups. The conclusion is that delays of 2 and 4 hours in CBC testing did not significantly affect leukocyte and platelet counts in Dengue Fever patients. These findings suggest that short-term delays in sample analysis may not compromise hematological results in DF diagnosis.





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## 1. INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is an acute infectious disease caused by the dengue virus (Adrizain, Setiabudi & Chairulfatah, 2019). Dengue Hemorrhagic Fever is one type of arboviral disease. When a mosquito bites a human during the viremic phase, the virus can replicate within the mosquito's body until the incubation period ends. Dengue Hemorrhagic Fever has become endemic in over 100 countries within the WHO region, with Asia accounting for approximately 70% of the global disease burden. In 2022, cases of Dengue Hemorrhagic Fever in Indonesia reached 143,000. According to records from the Bali Provincial Health Office, there were 2,469 cases of Dengue Hemorrhagic Fever (DHF) in the first three months of 2023. In Denpasar City alone, there were 1,096 reported cases as of July 2023 (Muliantari, 2023).

The main pathophysiology determines the severity of the disease and distinguishes Dengue Hemorrhagic Fever from classical dengue infection. Distinguishing features of classical dengue infection include increased vascular permeability, plasma leakage, hypotension, thrombocytopenia, and hemorrhagic diathesis. The exact pathophysiological mechanism of DHF remains unclear; however, most believe in the "secondary heterologous infection hypothesis," which states that DHF may occur after a primary dengue infection, followed by a secondary infection with a different dengue virus serotype within six months to five years (Pambudi, 2018).

To diagnose dengue infection, in addition to physical examination and medical history, additional tests such as complete blood count (CBC) are performed. These tests include parameters such as hemoglobin (HB), hematocrit (HCT), leukocytes (WBC), platelets (PLT), erythrocytes (RBC), MCV, MCH, and MCHC. Platelet counts typically remain normal during the first three days but begin to drop several days after the fever onset, eventually reaching a critical low during the shock phase. Thrombocytopenia, increased leukocyte (WBC) counts during the shock phase is another clinical diagnostic criterion. Among the CBC parameters, leukocyte and platelet counts are of particular diagnostic concern. Leukocyte counts in DHF patients can be normal or decreased. By the third day, relative lymphocytosis (less than 45% of total leukocytes) and the presence of blue plasma lymphocytes (less than 15% of total leukocytes) may be observed (Kudsiyah, 2020). A common pre-analytical issue in laboratories is the delay in testing, often due to neglecting Standard Operating Procedures (SOP). Test delays may occur when patient sample volumes exceed laboratory capacity while staffing remains insufficient, resulting in postponed testing (Ramadhani et al., 2019).

Previous research has shown that delays in hematology testing affect several parameters. MCV increases after 4 hours at 20°C and exceeds the threshold after 6 hours at 30°C. Basophil counts are difficult to interpret due to their low number and changing morphology over time. Automated cell analysis delayed beyond 4 hours may alter results and require manual verification. Other studies found that red blood cells, platelets, hemoglobin, and MCH remain stable for up to 48 hours at 4°C, 10°C, or 23°C, while hematocrit and MCV increase and white blood cells decrease after 48 hours at 23°C. Lymphocytes, neutrophils, eosinophils, and basophils show significant changes after 12 hours at 23°C (Yilmaz, et al., 2021). This study aims to determine the effect of leukocyte and platelet count results under delayed complete blood count (CBC) testing in patients with Dengue Hemorrhagic Fever. To support this research, samples must be collected from patients diagnosed with Dengue Hemorrhagic Fever.

## 2. METHOD

This research is an experimental study involving controlled trials to examine the impact of a specific treatment on the samples. This study uses a Posttest Only Control

Group Design, which consists of two groups: the first group receives treatment (experimental group), and the second group does not (control group). The research was conducted at the laboratory of Wangaya Regional Hospital, Denpasar.

Primary data were obtained through direct CBC testing, including delayed testing at 2 and 4 hours in DHF patients. Data collection was performed by conducting CBC tests immediately, after 2 hours, and after 4 hours at room temperature. The Shapiro-Wilk test was used to assess data normality. If data were normally distributed, a MANOVA test was conducted. If differences were found, post hoc testing was performed using the Bonferroni test (for homogeneous data) or the Games-Howell test (for non-homogeneous data).

Tools and materials used included stationery, tourniquet, syringe, vacutainer, plaster, timer, room thermometer, refrigerator, and hematology analyzer. Materials included alcohol swabs and EDTA tubes. Venous blood collection procedure started with explaining the study and obtaining written consent from the patient. Equipment was prepared, and the patient was asked to straighten their arm. A tourniquet was applied 7–10 cm above the cubital vein, and the patient was instructed to clench their fist. The vein was located and the area cleaned with 70% alcohol for 30 seconds. The needle was inserted at an angle of less than 30 degrees, and blood was collected into a citrate-anticoagulant tube. The tourniquet was released, and the patient relaxed their hand. The blood was gently inverted 5–10 times to mix. A cotton swab was applied to the puncture site, the needle was removed, and the site was covered with plaster. The patient's name, age, and gender were labeled on the EDTA tube. The workspace was cleaned and medical waste disposed of appropriately.

CBC analysis using the hematology analyzer: First, plug in and power on the device. After the device completes its self-check and is ready, ensure the blood sample is properly mixed. Press the "whole blood" button and assign the sample identification number. Open the sample holder, place the sample, and press "RUN" to begin testing. The results will be displayed automatically on the screen after a short moment.

## 3. RESULTS

**Table 1.** Leukocyte and Platelet Count Results with 0-Hour Examination Delay

| Sample Code | Leukocyte Count (×10³/µL) | Platelet Count (×10³/µL) |
|---|---|---|
| A01 | 3.75 | 112 |
| A02 | 4.47 | 91 |
| A03 | 5.32 | 44 |
| A04 | 6.93 | 44 |
| A05 | 6.34 | 12 |
| A06 | 2.22 | 132 |
| A07 | 2.20 | 22 |
| A08 | 2.87 | 35 |
| A09 | 9.72 | 62 |
| Average | 4.86 | 61.55 |

Table 1 shows that the average leukocyte count for immediate examination was 4.86×10³/µL**,** and the average platelet count was 61.55×10³/µL.

**Table 2.** Leukocyte and Platelet Count Results with 2-Hour Examination Delay

| Sample Code | Leukocyte Count (×10³/µL) | Platelet Count (×10³/µL) |
|---|---|---|
| B01 | 3.73 | 125 |
| B02 | 4.46 | 96 |
| B03 | 5.24 | 55 |
| B04 | 6.66 | 41 |

| | | |
|---|---|---|
| B05 | 6.14 | 10 |
| B06 | 2.10 | 149 |
| B07 | 2.11 | 21 |
| B08 | 2.81 | 35 |
| B09 | 9.85 | 60 |
| Average | 4.79 | 65.77 |

Table 2 shows that the average leukocyte count after a 2-hour delay was 4.79×10³/µL**,** and the average platelet count was 65.77×10³/µL.

**Table 3.** Leukocyte and Platelet Count Results with 4-Hour Examination Delay

| **Sample Code** | **Leukocyte Count (×10³/µL)** | **Platelet Count (×10³/µL)** |
|---|---|---|
| C01 | 3.88 | 123 |
| C02 | 4.52 | 98 |
| C03 | 5.23 | 53 |
| C04 | 6.64 | 40 |
| C05 | 6.20 | 11 |
| C06 | 2.25 | 132 |
| C07 | 2.15 | 25 |
| C08 | 2.83 | 37 |
| C09 | 9.97 | 57 |
| Average | 4.85 | 64.00 |

Table 3 shows that the average leukocyte count after a 4-hour delay was 4.85×10³/µL, and the average platelet count was 64.00×10³/µL. This study shows that the leukocyte count in the immediate examination group was 0.07×10³/µL higher than the leukocyte count in the 2-hour delayed group, which had a mean value of 4.79×10³/µL, indicating a decrease. In comparison, the leukocyte count in the 4-hour delayed group, with a mean of 4.85×10³/µL, showed an increase of 0.06×10³/µL. The platelet count showed an increase of 4.22×10³/µL in the 2-hour delayed group compared to the immediate examination group, which had a mean platelet count of 61.55×10³/µL. Compared to the 4-hour delay group, which had a mean of 64×10³/µL, there was a decrease of 1.77×10³/µL.

**Table 4**. Normality Test Results

| **Parameter** | **Delay Time** | **Shapiro-Wilk Test** | |
|---|---|---|---|
| | | **df** | **Sig. (p-value)** |
| Leukocyte Count | Immediate (0 hours) | 9 | 0.463 |
| | 2-hour delay | 9 | 0.421 |
| | 4-hour delay | 9 | 0.398 |
| Platelet Count | Immediate (0 hours) | 9 | 0.435 |
| | 2-hour delay | 9 | 0.409 |
| | 4-hour delay | 9 | 0.277 |

Table 4 shows that the Shapiro-Wilk normality test results for both leukocyte and platelet counts across the three time intervals had significance values ($p > \alpha = 0.05$). Therefore, the data for leukocyte and platelet counts in all three treatments are normally distributed.

**Table 5.** Homogeneity Test Results

| Parameter | p-value |
|---|---|
| Leukocyte Count | 0.998 |
| Platelet Count | 0.900 |

Table 5 indicates that the homogeneity test results for leukocyte and platelet counts yielded significance values of 0.998 and 0.900**,** respectively, both of which are greater than α (0.05)**.** This means the data in this study have homogeneous variance.

**Table 6.** MANOVA Test Results

| Parameter | Delay Time | df | Sig. (p-value) |
|---|---|---|---|
| Leukocyte Count | All groups | 9 | 1.000 |
| Platelet Count | All groups | 9 | 1.000 |

Table 6 shows that the multivariate analysis of variance (MANOVA) for complete blood count delay on leukocyte and platelet parameters resulted in a p-value of 1.000. Since $p > \alpha$ (0.05)**,** it can be concluded that there is no significant difference in leukocyte and platelet counts due to examination delay.

## DISCUSSION

The use of anticoagulants also needs to be considered in complete blood count examinations. Improper use of anticoagulants, such as heparin, is not recommended for complete blood count tests because it can cause blood cells to clump together, leading to invalid results. EDTA (Ethylenediaminetetraacetic Acid) anticoagulant is the most commonly used anticoagulant for complete blood count examinations. EDTA anticoagulant helps maintain the structure of blood cells and prevents platelet aggregation (Devi et al., 2024). Thrombocytopenia commonly occurs on the third day, suppressing platelet production in the bone marrow and leading to shortened platelet lifespan. In dengue infection patients, thrombocytopenia can occur through mechanisms such as bone marrow suppression, platelet destruction, and shortened platelet lifespan (Marpaung, Jayanti & Saragih, 2024).

Blood samples with anticoagulants that are not immediately examined can cause morphological changes in blood cells. Platelet metabolism remains active when stored at room temperature. Delayed examination leads to platelet aggregation and swelling, forming fragments with smaller sizes that cannot be counted as platelets by analyzers (Puspitasari, 2023). EDTA blood samples delayed for examination between 1–3 hours can cause swelling of leukocyte nuclei, leading to changes in cell integrity. Leukocytes undergoing disintegration will swell, enlarge, and become unreadable by hematology analyzers. Such morphological changes in leukocytes can interfere with leukocyte count, causing falsely low results (Puspitasari, 2023).

In contrast, results with a 4-hour delay in examination in this study show that after a 2-hour delay, platelet counts increased. This increase may be due to factors such as secondary or reactive thrombocytosis triggered by inflammatory responses. Inflammatory responses, such as vascular injury, stimulate the immune system, leading to increased platelet production for wound repair and healing. Other factors include heavy physical activity, like excessive exercise, which can transiently increase platelet count. Physical activity causes platelet aggregation, enhancing their adhesiveness (Putri et al., 2023). In a study by Ente et al., (2022), delayed platelet count examinations beyond 1 hour showed significant decreases. EDTA blood samples delayed for 1–3 hours caused swelling of leukocyte nuclei, chromatin changes, and cell disintegration. Meanwhile, platelet aggregation or adhesion occurred in samples delayed for 1 hour (Ente et al., 2023).

Platelet count decreases in delayed blood samples can occur due to several mechanisms. These include coagulation and clotting, where platelets activate coagulation processes when blood is left in collection tubes without special treatment, leading to reduced platelet numbers. Another mechanism is degradation, where enzymes and molecules in the blood cause platelet degradation during delays, altering their morphology and function, reducing their ability to be accurately counted. Lastly, cell lysis can occur, where blood cells, including platelets, break down due to factors like temperature changes, pH alterations, or chemical exposure, leading to decreased platelet counts upon examination (Putri, 2023).

Blood sample collection for platelet count should be performed promptly, ideally within one hour. Delayed examinations can cause platelet aggregation and swelling, forming fragments that cannot be detected as platelets by hematology analyzers (Devi, Rohmah & Astuti, 2024). Delays in examination can result from various factors, including equipment malfunctions, shift changes, power outages, sample transportation delays, and limited laboratory personnel. These factors affect the final platelet count results (Ente et al, 2022).

Statistical tests in this study indicated no significant effect of delays between immediate examination and 2-hour and 4-hour delays. However, mean values showed a decrease in leukocyte count at 2-hour delays and platelet count at 4-hour delays. This is due to leukocyte disintegration and platelet morphological changes during the delay periods. Puspitasari's (2022) study found no significant effect of delay duration on leukocyte count with a p-value of 0.954 (>0.05), but noted decreased leukocyte counts after 24-hour delays compared to immediate examinations (Puspitasari, 2023).

Blood samples stored at room temperature can cause platelet aggregation and adhesion, leading to decreased platelet counts. However, when stored in a refrigerator at 4–8°C, platelet metabolism is slowed, preventing aggregation and adhesion, thus stabilizing platelets. Blood specimens stored at room temperature (18–25°C) or refrigerated (4–8°C) for up to 24 hours can yield reliable results for complete blood count examinations. Platelet counts are typically stable for 8 hours after blood collection, but the optimal examination time is within 2 hours. Immediate examination after blood collection is recommended, as delays can cause cell lysis and bacterial growth (Hamadi, Irfani, & Shafriani, et al., 2021).

Anticoagulant use also affects leukocyte count averages in K2EDTA and K3EDTA blood samples. Excessive anticoagulant use can alter neutrophil morphology, causing cell swelling, loss of lobes, and disintegration into smaller fragments, leading to falsely low leukocyte counts. Decreased leukocyte counts can result from cell crenation, occurring when the anticoagulant-to-blood ratio is incorrect, causing blood hypertonicity. In this condition, blood maintains its osmotic pressure by drawing fluid out of cells, causing leukocytes to shrink and reducing their numbers (Maulin & Irma, 2023).

In the critical phase of dengue fever, increased capillary permeability can lead to plasma leakage. The critical phase in dengue hemorrhagic fever patients is characterized by leukocyte counts below $2 \times 10^3/\mu L$ and platelet counts below $2 \times 10^3/\mu L$. The severity of dengue hemorrhagic fever is classified into four degrees: degree 1 includes positive tourniquet test; degree 2 includes degree 1 plus spontaneous bleeding; degree 3 includes degrees 1 and 2 plus circulatory failure and weak pulse; degree 4 includes degree 3 with profound shock, absent pulse, and irregular blood pressure (Maulin & Irma, 2023).

This study also found unstable results, with increased leukocyte and platelet counts. Putri's (2023) study reported increased leukocyte counts immediately with a mean of $6.3 \times 10^3/\mu L$, and after 6 hours, the mean was $6.4 \times 10^3/\mu L$. This may be due to differences in sample homogenization during measurement, unmeasured delay temperatures, and

varying analyzer conditions between immediate and delayed examinations. Additionally, the relatively small sample size in this study may affect data uniformity (Merta, Artini & Subekti, 2014).

## 4. CONCLUSION

The conclusion of this study shows that delaying complete blood count, both for platelets and leukocytes, can affect the results obtained, with significant changes in blood cell morphology, such as platelet aggregation and swelling and leukocyte cell disintegration. Delays of up to 2 hours can cause an increase in platelet counts, while longer delays, up to 4 hours, can cause a decrease in platelet and leukocyte counts. Therefore, it is recommended that blood tests be performed immediately after sampling to ensure accurate results. If the test cannot be performed immediately, storing blood samples at a temperature of 4–8°C can help maintain platelet stability and prevent changes in cell morphology.

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