



DIFFERENCES IN PLATELET COUNTS USING BLOOD SAMPLES WITH K₃EDTA AND 3.2% SODIUM CITRATE ANTICOAGULANTS IN DENGUE FEVER PATIENTS

PERBEDAAN JUMLAH TROMBOSIT MENGGUNAKAN SAMPEL DARAH DENGAN ANTIKOAGULAN K₃EDTA DAN NATRIUM SITRAT 3,2% PADA PASIEN DEMAM BERDARAH

Anak Agung Istri Ari Trisnaningrum^{1*}, Ni Luh Nova Dilisca Dwi Putri², Putu Ayu Parwati³

^{1,2} Medical Laboratory Technology Study Program Diploma, STIKES Wira Medika Bali

³ Applied Bachelor's Degree Program in Medical Laboratory Study Program STIKES Wira Medika Bali

*email Koresponden: @ajungari1990@gmail.com

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Abstract

Dengue Hemorrhagic Fever (DHF) is a viral infection marked by a significant decrease in platelet count. Accurate platelet count testing is crucial for diagnosing and monitoring DHF patients. The pre-analytical phase is a critical stage in laboratory testing, where errors can significantly impact test outcomes. Anticoagulants, such as K₃EDTA and 3.2% sodium citrate, are commonly used in routine hematology tests to prevent blood clotting by binding calcium ions and inhibiting clotting factor activation. This study aimed to evaluate the difference in platelet counts between blood samples collected with K₃EDTA and 3.2% sodium citrate anticoagulants in DHF patients. An analytical observational study with a cross-sectional design was conducted involving 50 purposively sampled DHF patients. Statistical analysis yielded a p-value of 0.615 ($p > 0.05$), indicating no significant difference in platelet counts between the two anticoagulants. It can be concluded that the use of K₃EDTA and 3.2% sodium citrate does not significantly affect platelet count results in DHF patients. Future studies are recommended to involve larger sample sizes and consider microscopic examination methods, such as peripheral blood smears, to assess platelet aggregation visually.

Keywords: Anticoagulants, K₃EDTA, Platelets, 3.2% Sodium Citrate



Abstrak

Demam Berdarah Dengue (DBD) merupakan penyakit infeksi virus yang ditandai dengan penurunan jumlah trombosit secara signifikan, sehingga pemeriksaan trombosit yang akurat sangat diperlukan dalam proses diagnosis dan pemantauan pasien. Salah satu faktor penting yang memengaruhi hasil pemeriksaan laboratorium adalah penggunaan antikoagulan pada tahap pra-analitik. Penelitian ini bertujuan untuk mengetahui perbedaan jumlah trombosit pada pasien DBD berdasarkan jenis antikoagulan yang digunakan, yaitu K3EDTA dan natrium sitrat 3,2%. Jenis penelitian ini adalah penelitian observasional analitik dengan desain cross-sectional dengan jumlah sampel sebanyak 50 pasien, dengan teknik pengambilan sampel secara purposive sampling. Hasil analisis statistik menunjukkan nilai p-value sebesar 0,615 ($p > 0,05$), yang mengindikasikan bahwa tidak terdapat perbedaan yang signifikan antara hasil pemeriksaan trombosit menggunakan antikoagulan K3EDTA dan natrium sitrat 3,2%. Dengan demikian, dapat disimpulkan bahwa kedua jenis antikoagulan tersebut tidak memberikan perbedaan bermakna terhadap hasil hitung trombosit pada pasien DBD. Disarankan agar penelitian selanjutnya melibatkan jumlah sampel yang lebih besar dan menggunakan metode mikroskopis, seperti apusan darah tepi, untuk mengidentifikasi kemungkinan agregasi trombosit secara visual.

Kata kunci: Antikoagulan, K3EDTA, Natrium Sitrat 3,2%, Trombosit..

1. INTRODUCTION

Dengue Hemorrhagic Fever (DHF) remains a major public health concern in Indonesia. This disease is transmitted by the *Aedes aegypti* and *Aedes albopictus* mosquitoes, which serve as vectors for the dengue virus. One of the characteristic clinical features of DHF is a decrease in platelet count (thrombocytopenia), which increases the risk of bleeding and serious complications (Bahar et al., 2023). Thrombocytopenia is a condition in which the platelet count falls below the normal range, typically defined as a count lower than $140\text{--}150 \times 10^3/\mu\text{L}$. It can result from various causes such as viral infections, dengue fever, malaria, human immunodeficiency virus (HIV), malignancies, disseminated intravascular coagulation (DIC), aplastic anemia, immune thrombocytopenic purpura (ITP), or more severe systemic conditions such as extracorporeal membrane oxygenation (ECMO) (Wijaya et al., 2023).

Thrombocytopenia can also be caused by pre-analytical factors during blood sample examination, as well as the immune system's response, in a condition known as EDTA-dependent pseud thrombocytopenia. This is a laboratory diagnosis with an incidence rate of 0.1–2% (Wijaya et al., 2023). Pseud thrombocytopenia refers to a phenomenon where automated cell counters report a falsely low platelet count compared to the actual number. In pseud thrombocytopenia, the actual platelet count is within the normal range, but due to factors such as platelet aggregation, adhesion to other blood cells (e.g., leukocytes), or the presence of abnormally large platelets (giant platelets), the hematology analyzer fails to identify them accurately, resulting in an apparently low platelet count (Kurniawan, 2015). Routine hematology examinations, especially platelet counts, are essential for diagnosing and monitoring the condition of DHF patients (Gusnelly, 2023). However, the accuracy of laboratory test results is significantly influenced by pre-analytical factors, including the type of anticoagulant used. Errors at this stage can account for up to 60–70% of total laboratory errors (Siregar, 2018).



The pre-analytical phase refers to all procedures conducted before the sample is processed by an autoanalyzer. This includes specimen collection, handling, and anticoagulant selection all of which are critical to ensuring reliable results (Syuhada et al., 2021). The recommended anticoagulants for routine hematological testing are Ethylenediaminetetraacetic acid (EDTA) and 3.2% sodium citrate (Gusnelly, 2023). Both EDTA and 3.2% sodium citrate work by chelating calcium ions into calcium salts, thereby inhibiting the coagulation cascade by preventing calcium from activating clotting factors (Rizka & Nugraha, 2023). Previous studies by Diba et al. (2019) and Devi et al. (2016) reported that platelet counts obtained from citrate samples were lower than those from EDTA samples. Other studies by Dima et al. (2020) and Artati (2021) focusing on patients suspected of having pseudothrombocytopenia also found significantly lower platelet counts in EDTA tubes compared to sodium citrate tubes, demonstrating a statistically significant difference in platelet count results.

At Prima Medika General Hospital, low platelet count results are frequently confirmed by re-sampling using 3.2% sodium citrate tubes. This underscores the need for a study examining the effect of using K3EDTA and 3.2% sodium citrate anticoagulants on platelet count results in DHF patients to ensure diagnostic accuracy in the laboratory. This study aims to determine the difference in platelet counts between blood samples collected using K3EDTA and 3.2% sodium citrate anticoagulants in dengue patients. A better understanding of these differences is expected to enhance awareness and knowledge regarding appropriate anticoagulant use in DHF patients, thereby supporting accurate patient diagnosis.

2. RESEARCH METHOD

This research is an analytical observational study with a cross-sectional design aimed at evaluating differences in platelet counts between blood samples collected in K3EDTA and 3.2% sodium citrate anticoagulants in dengue patients (Abduh et al., 2023). The study was conducted at the Clinical Pathology Laboratory of Prima Medika General Hospital, Denpasar, in February 2025. The study population was divided into two categories: the target population, which includes all suspected DHF patients in Bali, and the accessible population, consisting of suspected DHF patients from the outpatient and inpatient departments of Prima Medika General Hospital who met the inclusion and exclusion criteria. The sampling technique used was accidental sampling, involving respondents who were willing to participate in the study, totaling 50 patients. Exclusion criteria included patients diagnosed with dengue or febrile illness on day 1 or 2, patients with a history of hematological disorders associated with thrombocytopenia, samples that experienced clotting, and those with insufficient volume at the time of collection. Data analysis was performed using SPSS version 30. The analysis began with normality testing (Shapiro-Wilk test), homogeneity testing (Levene's test), and comparative testing using the Independent Samples T-Test. This study received ethical approval from the Health Research Ethics Committee of STIKES Wira Medika Bali, as evidenced by an ethical clearance letter with reference number 414/E1.STIKESWIK/EC/III/2025. All participants provided written informed consent, and all data were kept strictly confidential for academic purposes only.



3. RESULTS AND DISCUSSION

This study's findings are divided into three main sections: the normality test, the homogeneity test, and the comparative (difference) test. The characteristics of the respondents in this study are as follows:

Table 1
Characteristics of Respondents Based on Gender and Age

Characteristics	Number (People)	Percentage (%)
1. Gender		
a. Male	34	68
b. Female	16	32
Total	50	100
2. Age		
a. Toddler (0–5 years)	2	4
b. Children (5–11 years)	5	10
c. Early Adolescence (12–16 years)	8	16
d. Late Adolescence (17–25 years)	11	22
e. Early Adulthood (26–35 years)	14	28
f. Late Adulthood (36–45 years)	6	12
g. Early Elderly (46–55 years)	2	4
h. Late Elderly (56–65 years)	1	2
i. Old Age (≥ 65 years)	1	2
Total	50	100

The majority of respondents in this study were male (68%), while females accounted for 32%. In terms of age distribution, the largest group belonged to early adulthood (26–35 years), representing 28% of participants, followed by late adolescence (17–25 years) with 22%. Respondents from the toddler, late elderly, and elderly age groups each comprised the smallest proportions, ranging from 2% to 4% of the total sample. Overall, most respondents were adults, while older age groups were the least represented.

Table 2. Platelet Count Test Results



Anticoagulants	Mean ($10^3/\mu\text{L}$)	SD ($10^3/\mu\text{L}$)
K ₃ EDTA	65,44	22,384
Sodium citrate 3,2%	63,2	22,035

Based on Table 2, the platelet count test results indicate a difference in the mean values between the two types of anticoagulants used: K₃EDTA and 3.2% sodium citrate. Blood samples collected with K₃EDTA showed a mean platelet count of $65.44 \times 10^3/\mu\text{L}$ with a standard deviation (SD) of $22.384 \times 10^3/\mu\text{L}$. In contrast, samples using 3.2% sodium citrate had a mean platelet count of $63.20 \times 10^3/\mu\text{L}$ with a standard deviation of $22.035 \times 10^3/\mu\text{L}$. These findings suggest that platelet counts tend to be slightly higher in samples using K₃EDTA compared to those using 3.2% sodium citrate.

Normality and homogeneity tests were subsequently performed. The results of the Shapiro-Wilk normality test showed p-values of 0.423 for K₃EDTA and 0.421 for 3.2% sodium citrate, both greater than 0.05, indicating that the data were normally distributed. Homogeneity testing using Levene's test yielded a p-value of 0.879. Since $p > 0.05$, the data sets are considered to have equal variances, indicating homogeneity across the groups. Given that the data are both normally distributed and homogeneous, an Independent Samples T-Test was conducted to assess whether there was a statistically significant difference between the two groups. The results are presented in the following table:

Table 3. Data Analysis Results

Variable	<i>p-value</i>
Difference in anticoagulant results of K ₃ EDTA and Sodium citrate 3.2%	0,615

Based on the results of the Independent Samples t-test, a p-value of 0.063 was obtained. Since the p-value is greater than the significance level ($p > 0.05$), it can be concluded that there is no statistically significant difference in platelet count between blood samples collected using K₃EDTA and those using 3.2% sodium citrate.

Discussion

The platelet count examination in DHF patient blood samples showed that the mean result using K₃EDTA anticoagulant was $65.44 \times 10^3/\mu\text{L}$, while the mean result using 3.2% sodium citrate was $63.20 \times 10^3/\mu\text{L}$. Although there was a slight difference in the mean values, the statistical analysis showed a p-value of 0.615 ($p > 0.05$), indicating no significant difference between the use of K₃EDTA and 3.2% sodium citrate anticoagulants.

The slightly higher mean platelet count observed in samples using K₃EDTA may be attributed to its superior platelet stabilization capability. EDTA irreversibly binds to calcium ions (Ca^{2+}), effectively and permanently preventing blood coagulation and maintaining the stability of blood cell components, including platelets, for a longer period after blood collection. EDTA provides more accurate results for platelet and other cell counts, as it does not induce dilution effects, unlike sodium citrate (Dima et al., 2020).



The use of EDTA as an anticoagulant in routine hematology tests does not significantly dilute the blood sample. This is due to the small volume of EDTA used, which is proportionally adjusted to the blood volume in the tube. The volume of anticoagulant in EDTA tubes is 0.02 mL for 2 mL of blood (ratio 1:100) (Devi et al., 2016).

In contrast, blood samples using 3.2% sodium citrate showed a slightly lower mean platelet count. This may be due to the reversible nature of sodium citrate's calcium binding and its dilution effect (Dima et al., 2020). The anticoagulant-to-blood ratio in sodium citrate tubes is 0.2 mL anticoagulant to 1.8 mL blood (ratio 1:9), which contributes to the dilution (Devi et al., 2016).

Despite these differences in anticoagulant properties, the statistical results showed no significant difference in platelet counts between the two groups. This may be explained by the similar mechanisms of both anticoagulants, which function by binding calcium ions to form inactive calcium salts, thereby preventing the activation of coagulation factors (Rizka & Nugraha, 2023). The absence of a statistically significant difference in this study may also be attributed to the specific characteristics of DHF, where thrombocytopenia is a well-known clinical manifestation. This typically occurs between days 3 to 7 of infection, when platelet counts can fall to $\leq 100,000/\mu\text{L}$ (Ministry of Health of the Republic of Indonesia, 2017).

These findings are consistent with previous studies by Diba et al. (2019) and Devi et al. (2016), which also found lower platelet counts in samples using sodium citrate compared to EDTA. Thus, both K_3EDTA and 3.2% sodium citrate can be used for platelet count measurement. However, K_3EDTA is the recommended anticoagulant for routine hematological analysis. In certain cases especially when low platelet counts are reported or pseudothrombocytopenia is suspected result verification should be performed using sodium citrate anticoagulant or by microscopic confirmation to detect platelet aggregation, thereby improving diagnostic accuracy.

4. CONCLUSION

Based on the research findings and data analysis involving 50 patients with dengue hemorrhagic fever, the following conclusions can be drawn: Platelet count using K_3EDTA anticoagulant yielded a mean value of $65.44 \times 10^3/\mu\text{L}$ with a standard deviation (SD) of $22.384 \times 10^3/\mu\text{L}$; Platelet count using 3.2% sodium citrate showed a lower mean value of $63.20 \times 10^3/\mu\text{L}$ with a standard deviation of $22.035 \times 10^3/\mu\text{L}$; There was no statistically significant difference in platelet count between samples collected with K_3EDTA and 3.2% sodium citrate anticoagulants, as indicated by the statistical test result with a p-value of 0.615 ($p > 0.05$).

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