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CORRELATION BETWEEN THROMBOCYTOPENIA DEGREE AND PARASITE DENSITY IN CONFIRMED CASES OF *Plasmodium falciparum* AND *Plasmodium vivax* MALARIA

KORELASI DERAJAT TROMBOSITOPENIA DAN KEPADATAN PARASIT PADA KASUS TERKONFIRMASI MALARIA Plasmodium falciparum DAN Plasmodium vivax

Original Research Article
Penelitian

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A B S T R A C T

Background: *Thrombocytopenia, a condition characterized by a low platelet count, is the most prevalent hematological abnormality observed in acute malaria patients. Malaria remains a major global public health problem, with more than 200 million clinical cases reported annually.* ***Purpose:*** *This study aimed to investigate the correlation between the degree of thrombocytopenia and the parasite density in confirmed cases of Plasmodium falciparum and Plasmodium vivax.* ***Method:*** *This research was a descriptive observational study using a cross-sectional design. Clinical hematological examinations and peripheral blood smear preparations were performed on malaria patients, followed by analysis of platelet count, hemoglobin levels, and leukocyte count.* ***Result:*** *Thrombocytopenia, commonly found in acute malaria, was observed in 63.4% of cases, underscoring its key role as a diagnostic biomarker. This study showed significant association between hemoglobin levels and thrombocytopenia severity (p-value < 0.05), whereas leukocyte counts did not show a significant association with thrombocytopenia severity (p-value > 0.05). The degree of thrombocytopenia differed between the two types of malaria, assisting the differentiation of infections. Anemia, another detailed hematological indicator, frequently found in P. falciparum cases.* ***Conclusion:*** *Understanding hematological indicator as key-role of malaria diagnosis is vital for accurate diagnosis and effective management of malaria, especially in endemic regions. Continued research and routine hematological surveillance are crucial to improving malaria control and treatment outcomes.*

A R T I C L E I N F O

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A B S T R A K

Latar belakang: Trombositopenia, yaitu kondisi yang ditandai dengan rendahnya jumlah trombosit, merupakan suatu kondisi hematologi yang sering terjadi pada pasien malaria akut. Malaria tetap menjadi tantangan besar dalam kesehatan masyarakat global, dengan lebih dari 200 juta kasus klinis dilaporkan setiap tahunnya. **Tujuan:** Penelitian ini bertujuan untuk menyelidiki hubungan antara derajat trombositopenia dengan kepadatan parasit *Plasmodium falciparum* dan *Plasmodium vivax*. **Metode:** Penelitian ini merupakan studi observasional deskriptif dengan desain *cross-sectional*. Pemeriksaan hematologi klinis dan preparasi hapusan darah tepi dilakukan pada pasien malaria, yang kemudian dianalisis terhadap jumlah trombosit, kadar hemoglobin, dan jumlah leukosit. **Hasil:** Trombositopenia yang umum ditemukan pada malaria akut, terdeteksi pada 63,4% kasus, menunjukkan pentingnya trombosit sebagai biomarker diagnostik. Penelitian ini menunjukkan hubungan yang signifikan antara kadar hemoglobin dan derajat trombositopenia, sedangkan hubungan jumlah leukosit dan derajat keparahan trombositopenia menunjukkan hasil tidak signifikan (*p-value* > 0,05). Derajat trombositopenia berbeda antara kedua jenis malaria tersebut, sehingga dapat membantu dalam membedakan jenis infeksi. Anemia, sebagai kelainan hematologi lain yang juga umum, lebih menonjol pada kasus *P. falciparum*, yang menunjukkan signifikansi klinisnya. **Kesimpulan:** Indikator hematologi sebagai poin penting dalam diagnosis malaria diperlukan untuk diagnosis yang akurat dan penanganan malaria yang efektif. Penelitian lebih lanjut dan pengawasan rutin sangat diperlukan untuk upaya pencegahan dan penanggulangan malaria di daerah endemis.

Kata kunci:
Malaria, Kepadatan Parasit, *Plasmodium falciparum*, *Plasmodium vivax*, Trombositopenia

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INTRODUCTION

Malaria is an infectious disease associated with varying degrees of hematological complications with anemia and thrombocytopenia being the most common manifestations (Sharma *et al*., 2017). According to the World Health Organization (WHO), approximately 40% of the global population is at risk of malaria infection. The annual incidence of malaria reported among 300 to 500 million cases, reached two million deaths each year (Arif *et al*., 2016). In Indonesia, there are five species of malaria exist, *Plasmodium falciparum* (*P. falciparum*), *Plasmodium vivax* (*P. vivax)*, *P. malariae*, *P. ovale*, and *P. knowlesi* (Zein *et al*., 2017).

Early clinical detection of malaria is critical for its successful treatment. Diagnosis is typically performed using light microscopy to examine thick and thin blood smears, which remains the most reliable and widely applicable method for detecting malaria. The severity of malaria is associated to the parasitemia degree, with high levels of parasitemia resulting in severe malaria (Antwi-Baffour *et al*., 2023). Severe malaria is defined by the presence of asexual stage of malaria parasites, accompanied by serious symptoms or complications of malaria (Naing and Whittaker, 2018). Severe *P. falciparum* malaria is obvious shown on clinical manifestations, including decreased consciousness, multiple convulsions, acute respiratory distress, circulatory collapse or shock, acute kidney injury, prostration, clinical jaundice, and abnormal bleeding. A study conducted in Bangkok showed lower platelet count in cases of severe malaria compared to malaria without serious complications (Mon *et al*., 2022).

According to the latest WHO guidelines, hematological indices, including thrombocytopenia, are not considered criteria for defining severe malaria, however their clinical significance is widely recognized (Teparrukkul *et al*., 2019). A retrospective study involving 614 patients revealed that individuals with thrombocytopenia were more likely to develop Multi-Organ Dysfunction (MOD) and had an increased risk of mortality compared to malaria patients with normal platelet counts (Gill *et al*., 2013).

Thrombocytopenia is a known complication of malaria caused by *P. falciparum* infection. However, recent studies have shown that *P. vivax* show similar levels of severity (Bayleyegn *et al*., 2021). Hematological indices of malaria patients have reveal thrombocytopenia, which has been linked to excessive platelet sequestration and a shortened platelet lifespan (Lestari, 2019; Muley *et al*., 2014). The proposed mechanisms leading to thrombocytopenia include coagulation disturbances, splenomegaly, bone marrow alterations, antibody-mediated platelet destruction, oxidative stress, and the role of platelets as cofactors in triggering severe malaria (Kumar *et al*., 2022; Muley *et al*., 2014; Natalia, 2014).

This study aimed to assess the importance of thrombocytopenia and its potential role as an early diagnostic marker for malaria. The presence of thrombocytopenia can serve as a noticeable clinical indicator of malaria symptoms. In addition to, this study sought to establish a correlation between the presence of thrombocytopenia and the type of malaria in regions where malaria is endemic in Indonesia.

MATERIAL AND METHOD

This descriptive observational study employed a cross-sectional design. The research involved clinical hematological examinations and peripheral blood smears analyses of malaria patients visited a laboratory in Jayapura, which were then analyzed for platelet count, hemoglobin levels, and leukocyte count. Blood samples were collected from people with malaria who visited healthcare service units. The blood samples were stored in ethylenediaminetetraacetic acid (EDTA) tubes and used to prepare thin and thick blood films for giemsa staining and microscopic identification of malaria parasites and species. In this study, individuals with malaria were diagnosed through blood smear examination, and automated hematology analyzer used to assess hematological indicators. Trained medical laboratory scientists collected the blood samples, and blood counts were conducted using the Sysmex XP-100 hematology analyzer.

To discover the density of malaria parasites, a blood technician independently counted the asexual stage Plasmodium parasites on slides, specifically counting 200 White Blood Cells (WBCs) in thick blood films from each malaria case. The counts from two independent technician were then averaged, and this mean value was used to calculate the parasite density. The number of parasites per microliter (µL) of blood was then determined using the following Formula (1).

$$\text{Parasite}/\mu L = \frac{\text{parasite counted}}{200} \times \text{total WBC count} \quad \ldots. (1)$$

Platelet counts were determined by directly measuring platelet pulses and was expressed as thousands of platelets per microliter of whole blood. Thrombocytopenia was defined as a platelet count of less than 150.000/µL. Patients were categorized into three subgroups based on their platelet counts. Thrombocytopenia was considered severe if the platelet count was less than 50.000/µL, moderate if it ranged

from 50.000 to 100.000/µL, and mild if it fell between 100.000 and 150.000/µL (Kumar *et al*., 2022).

Malaria parasite density was determined using the aforementioned formula and was categorized as mild, moderate, or severe parasitemia. Blood samples with 5 – 10.000 parasites/µL could be considered as low parasitemia corresponding to mild malaria, and those with 10.000 – 100.000 parasites/µL were categorized as intermediate parasitemia indicating moderate malaria, whilst parasitemia above 100.000 parasites/µL was classified as hyper parasitemia and described as severe malaria which may lead to death (Wilairatana *et al*., 2013).

Thrombocytopenia and parasite density were treated as ordinal variables. To evaluate the correlation between them, the Spearman's rank correlation test was used. A p-value of less than 0.05 was considered statistically significant.

RESULT

A total of 41 eligible patients were enrolled and randomly assigned in the study, with their characteristics summarized in Table 1. Among these patients, 39% were diagnosed with *P. falciparum* malaria, while 61% had *P. vivax* malaria, as shown in Table 2. Thrombocytopenia was observed in 63% of the cases, with varying degrees of severity. The association between thrombocytopenia and the species of malaria showed no statistically significant difference (p-value > 0.05), as presented in Table 3. Mild thrombocytopenia was more frequently associated with *P. vivax* (52.9%) compared to *P. falciparum* (33.3%), whereas moderate thrombocytopenia was more commonly linked to *P. falciparum* (66.7%) than *P. vivax* (41.2%). However, these differences were not statistically significant (p-value > 0.05), also presented in Table 3.

Table 1. Demographic characteristic of malaria patients

Characteristic	Value	n	Frequency (%)
Age group (years)	18 – 24	5	12.19
	25 – 34	11	26.83
	35 – 44	14	34.15
	45 – 54	9	21.95
	55 – 64	2	4.88
	>65	0	0
Gender	Male	26	63.41
	Female	15	36.59
Education	High school	20	48.78
	Diploma/undergraduate	21	51.22

Table 2. Malaria parasite density according to malaria types

Malaria parasite density (parasites/µL)	Malaria type		Total
	Malaria *falciparum*	Malaria *vivax*	
Low	1 (6.25%)	3 (12%)	4 (9.75%)
Medium	13 (81.25%)	22 (88%)	35 (85.36%)
High	2 (12.5%)	0	2 (4.87%)
Total	16 (100%)	25 (100%)	41 (100%)

Table 3. Association between malaria types and thrombocytopenia severity

Malaria type	Thrombocytopenia (%)			p-value
	Mild	Moderate	Severe	
P. vivax	52.9	41.2	5.9	0.410
P. falciparum	33.3	66.7	0	

Table 4. Association between malaria parasite density and thrombocytopenia severity

Malaria parasite density (parasites/µL)	Thrombocytopenia (%)			p-value
	Mild	Moderate	Severe	
Low	0	100	0	0.185
Medium	57.1	38.1	4.8	
High	0	100	0	

Table 5. Association between hematological parameters and thrombocytopenia severity

Hematological parameter	Severity of thrombocytopenia (mean)				p-value
	Normal	Mild	Moderate	Severe	
Hemoglobin (g/dL)	12.667	12.678	12.463	12.632	0.002
Leukocytes (mm^3)	6553.33	5333.33	5350.00	5782.93	0.117

Table 2 presents the distribution of malaria patients according to parasite density. which was categorized into three levels based on WBC counts: (1) Low, (2) Medium, and (3) High density. High density parasitemia was observed only in *P. falciparum* cases, while no high-density cases were found in *P. vivax* infections.

Table 3 presents the distribution and statistical analysis of the association between malaria type and the severity of thrombocytopenia. The results indicate that there is no significant difference between *P. falciparum* and *P. vivax* in inducing thrombocytopenia. Thrombocytopenia severity is categorized into three groups based on platelet count: (1) Severe <50.000/µL, (2) Moderate 50.000 - 100.000/µL, and (3) Mild 100.000 -150.000/µL.

Table 4 presents the association between malaria parasite density and the severity of thrombocytopenia. Malaria parasite density is categorized based on WBC count, while thrombocytopenia severity is classified as mild, moderate, or severe based on platelet count. The statistical analysis showed a p-value > 0.05, indicating no significant association between parasite density and thrombocytopenia severity.

Table 5 presents two hematological indices, hemoglobin and leukocyte levels. Each parameter shows the mean values for individuals with malaria, categorized as normal or having mild, moderate, or severe thrombocytopenia. Hemoglobin levels demonstrated a statistically significant difference (p-value < 0.05), whereas leukocyte counts did not show a significant association with thrombocytopenia severity (p-value > 0.05).

DISCUSSION

Malaria remains a major global health concern in tropical regions, including Indonesia. Continues studies have been conducted in endemic areas such as Papua. Thrombocytopenia is frequently observed in cases of acute malaria and can occur in both *P. falciparum* and *P. vivax* infections. Platelet indices serve as valuable, cost-free biomarkers that can help diagnose malaria by reflecting platelet activation, which is important for clinical investigations into the condition's prognosis and severity (Bayleyegn *et al*., 2021).

The demographic characteristics of the study respondents can be seen in Table 1, showed a varied distribution in terms of age, gender, and educational background. Based on age group, the majority of participants were in the 35 – 44 years category 34.15%, followed by the 25 – 34 years group 26.83% and the 45 – 54 years group 21.95%. A smaller proportion of respondents belonged to the 18 – 24 years group 12.19% and 55 – 64 years group 4.88%. Notably, no respondents were aged over 65 years. These findings indicate that the respondents were generally in the productive age group, which may suggest their active involvement in and awareness of malaria-related issues.

In terms of gender, distribution was predominantly male 63.41%, while females comprised 36.59% of the total respondents. This imbalance may reflect the actual composition of the target population or suggest a gender bias in respondent availability or willingness to participate in the study. Regarding educational attainment, most respondents had either a high school education 48.78% or a diploma/undergraduate degree 51.22%. This relatively high level of education among the participants may influence their knowledge, attitudes, and practices related to malaria.

In the present study, 61% of the cases were *P. vivax* malaria, 39% were *P. falciparum* malaria, and no mixed infections were observed. A comparable study conducted by Khalid *et al.* (2022) reported that *P. vivax* and *P. falciparum* accounted for 90% and 10% of the cases, respectively. Gupta *et al*. (2019) also reported that 73% of the cases were *P. vivax*, 23% were *P. falciparum*, and no mixed infections were detected. Later, in 2015, Krishna and Chalamalasetty (2023) supported the present study with similar findings, showing that 42% of

the cases were *P. vivax* malaria, 24% were *P. falciparum* malaria, and the rest were mixed infections. The present study, along with the aforementioned studies, indicates that *P. vivax* malaria is the predominant species among all recorded cases. The high prevalence of *P. vivax* malaria in tropical regions can be attributed to climatic variations, the presence of suitable mosquito breeding habitats, and the genetic resistance to *P. falciparum*.

Through this study, the incidence of thrombocytopenia observed 63.4%. Similar findings have also reported thrombocytopenia to be common among malaria patients. Punnath *et al*. reported a similar incidence of 62.7%, Krishna and Chalamalasetty 81%, Awoke and Arota 84%, and Suryadi *et al*. 90% (Awoke and Arota, 2019; Krishna and Chalamalasetty, 2023; Punnath *et al*., 2019; Suryadi *et al*., 2021). The present findings, together with those from previous research, indicate that thrombocytopenia is the most frequent finding as clinical case of malaria.

In this study, mild thrombocytopenia was more frequently associated with *P. vivax* 52.9% than with *P. falciparum* 33.3%. Moderate thrombocytopenia was observed in both *P. falciparum* 66.7% and *P. vivax* 41.2% infections, whereas severe thrombocytopenia was more frequently associated with *P. vivax* 5.9% compared to *P. falciparum*. These findings are similar to those reported in study by Kumar *et al*. (2022), who reported that mild thrombocytopenia occurred in *P. vivax* 52.08% and *P. falciparum* 10.71% cases, while moderate thrombocytopenia was found in both *P. falciparum* 42% and *P. vivax* 40%. However, severe thrombocytopenia was more frequently linked to *P. falciparum* 46.43% than to *P. vivax* 8.34% (Kumar *et al*., 2022). Although thrombocytopenia is commonly observed in malaria, its absence is considered atypical. However, its presence does not serve as a reliable differentiating factor between *P. falciparum* and *P. vivax* malaria (Bayleyegn *et al*., 2021; Muley *et al*., 2014).

The severity of malaria correlates with the degree of parasitemia, where elevated levels of parasitemia lead to severe malaria (Antwi-Baffour *et al*., 2023). Accordingly, a blood sample containing 5 – 10.000 parasites/µL may indicate low parasitemia, resulting in mild malaria, while one with 10.000 – 100.000 parasites/µL may indicate intermediate parasitemia, resulting in moderate malaria. Parasitemia exceeding 100.000 parasites/µL are classified as hyperparasitemia and can lead to severe malaria, potentially resulting in fatality (Wilairatana *et al*., 2013). However, this study shows no association between malaria severity and parasite density.

Several observational studies have confirmed the correlation between thrombocytopenia and malaria. Thrombocytopenia in malaria has been attributed to both non-immunological and immunological mechanisms involving the destruction of platelet (Barada *et al*., 2023). Several mechanism like oxidative stress, immune responses, altered splenic function due to organomegaly, and direct interactions between the *Plasmodium* parasite and platelets impacted by the reduction of platelet usually observed (Haroon *et al*., 2021).

This study examined the association between hematological parameters (hemoglobin and leukocyte counts) and the severity of thrombocytopenia in *P. falciparum* and *P. vivax* malaria. The results revealed a statistically significant relationship between hemoglobin levels and the degree of thrombocytopenia (p-value = 0.002), while the association between leukocyte count and thrombocytopenia was not statistically significant (p-value = 0.117). The reduction in hemoglobin levels with increasing thrombocytopenia severity may be attributed to malaria-induced hemolysis, bone marrow suppression, and cytokine-mediated dysregulation. Those condition contribute to anemia in malaria patients. This finding aligns with previous research that reported a correlation between anemia and thrombocytopenia in malaria, particularly in *P. falciparum* infections (Gupta *et al*., 2019; Kumar *et al*., 2022). Hemolysis of both infected and uninfected erythrocytes, along with ineffective erythropoiesis, are mechanisms innitiate to decreased hemoglobin levels in malaria (Naing and Wittaker, 2018).

This study found that leukopenia was more frequently observed in patients with more severe thrombocytopenia however the association was not statistically significant. This result suggests leukocyte counts may fluctuate during malaria infection, they are not consistently associated with platelet depletion. Variations in leukocyte responses may be influenced by multiple factors, such as host immune status, co-infections, or the stage of parasitemia (Krishna *et al*., 2023; Punnath *et al*., 2019; Tabassum and Iqbal, 2021).

This findings are consistent with studies conducted in both endemic and non-endemic regions, reporting thrombocytopenia as a common hematological abnormality in malaria and often correlates with disease severity (Zein *et al*., 2017). However, the absence of a significant association with leukocyte counts contrasts with some previous research, highlighting the variability of leukocyte responses across different populations and *Plasmodium* species.

Malaria often presents with common symptom of high fever and various hematological abnormalities in a complete blood count, including anemia, thrombocytopenia, and leucopenia (Barada *et al*., 2023). The key contributing factors to anemia in malaria patients include coexisting conditions such as helminth infections, Human Immunodeficiency Virus (HIV) infection, and other protozoal infections (Costa *et al*., 2020; Muflikhah and Nuraini, 2023).

CONCLUSION

The findings of this study demonstrate that thrombocytopenia was present in 63.4% of acute malaria cases, emphasizing its significance as a diagnostic biomarker. Statistical analysis revealed a significant association between hemoglobin levels and the severity of thrombocytopenia (p-value < 0.05), indicating that lower hemoglobin concentrations are linked to more severe platelet reduction. In contrast, leukocyte counts showed no significant relationship with thrombocytopenia severity (p-value > 0.05), suggesting that white blood cell changes are less indicative of disease severity in malaria.

This study also provides valuable insights into the prevalence and hematological profiles, mainly thrombocytopenia and anemia, associated with different types of malaria infections. The diagnosis, prognosis, and effective management of malaria can easily understand though hematological marker detected during examination, especially in endemic regions like Papua, Indonesia. Further research and routine hematological surveillance are crucial for a wide-ranging understanding of malaria and hematological implications.

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The author declares no conflict of interest with any parties involved in this study.

AUTHOR CONTRIBUTION

The author contributed significantly to the conception, design, and execution of the study. The author developed the research framework, collected and analyzed the data, interpretation of results, manuscript drafting.

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DATA AVAILABILITY

The data supporting the findings of this study are available upon request from the author.

CONFLICT OF INTEREST

The author declares no conflicts of interest regarding the publication of this paper.

ETHICAL APPROVAL

Ethical approval was obtained under approval by Ethic Committee of Health Institute of Guna Bangsa number: 052/KEPK/VIII/2020 issued on August 2020.

INFORMED CONSENT

Informed consent was obtained from all individual participants included in the study. Participants were informed about the purpose, procedures, risks, and benefits of the research before data collection. Confidentiality and anonymity of the participants were strictly maintained throughout the study.

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