

Comparison Parasite Density and Count the Number of Reticulocytes in Patients with Falciparum Malaria and Vivax Malaria at the Sentani Community Health Center, in Jayapura District, Papua Province, Indonesia

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Submitted: 12 August 2025 Accepted: 22 August 2025 Published: 28 August 2025

doi <https://doi.org/10.63620/MKJMMRR.2025>.

Citation: Sorontou, Y., & Agussalim. (2025). Comparison Parasite Density and Count the Number of Reticulocytes in Patients with Falciparum Malaria and Vivax Malaria at the Sentani Community Health Center, in Jayapura District, Papua Province, Indonesia. *J of Med Ima & Med Edu Res* 2(4). 01-05.

Abstract

Background: Reticulocytes are young, non-nucleated red blood cells that originate from the maturation process of normoblasts in the bone marrow. Reticulocyte count is an important indicator of bone marrow response to anemia, including that caused by malaria. *Plasmodium falciparum* is the causative agent of falciparum malaria, while *P. vivax* causes tertian malaria. Both types are transmitted through the bite of female *Anopheles* mosquitoes and present with similar clinical symptoms such as fever, chills, nausea, vomiting, headache, and joint pain. However, they differ in the onset and pattern of fever.

Objective: This study aimed to examine the differences in reticulocyte count and parasite density of *P. falciparum* and *P. vivax* in patients with falciparum and tertian malaria, respectively, at Sentani Public Health Center.

Methods: This was a descriptive study using a cross-sectional design. A total of 75 patients diagnosed with malaria were included in the sample.

Results: In patients with falciparum malaria, increased reticulocyte counts were observed at parasitemia level +2 in 10(25%) patients, at level +3 in 6 (15%) patients, and at level +4 in 2(5%) patients. Meanwhile, in patients with tertian malaria (*P. vivax*), increased reticulocyte counts were observed starting at parasitemia level +1 in 4 (11.4%) patients, level +2 in 6(17%) patients, level +3 in 5(14.3%) patients, and level +4 in 5(14.3%) patients.

Conclusion: This study shows that in falciparum malaria, reticulocyte count increases were found starting from parasitemia levels +2 to +4. In contrast in patients infected with *P. vivax*, increased reticulocyte counts were observed across a wider range of parasitemia levels, from +1 to +4. These findings indicate a differing pattern of bone marrow response to parasitic infection in the two types of malaria.

Keywords: Plasmodium Falciparum, P. Vivax, Parasite Density, Reticulocyte, Sentani Community Health Center

Introduction

Malaria is still one of the major public health problems in the world. Base on world malaria report showing 247 million in news malaria cases (up from 245 million in 2020). The global malaria death toll was 619.000 (up from 625.000 in 2020) [1].

The 2023 world malaria report delves in to the nexus between climate change and malaria. Changes in temperature, humidity and rainfall can influence the behavior and survival of the malaria carrying *Anopheles* mosquito [1].

The estimated number of new malaria cases found in Indonesia

was 811.636 in 2021 with a malaria death in the rate of 1.412 cases and 86% of these malaria cases occurred in the Province of Papua, Central Papua and South Papua [2].

Reticulocytes are young red blood cells or immature erythrocyte. These cells are produced by the body through the bone marrow, then sent to the circulatory system, when they are mature in about 1 to 2 days. The number reticulocytes are about 1 % of the number of erythrocytes. The normal value of reticulocytes is 0.5 to 1.5 %. Reticulocytes are produced by the bone marrow in response to anemia [3].

The cause of the increase in the number of reticulocytes in the human body is 1). Erythrocyte rupture due to merozoites invading the erythrocytes, causing hemolytic anemia in patients, which is characterized by yellow eyes and skin, 2). Response to iron (Fe), vitamin B12 or folic acid, supplementation therapy and these supplements are given increase erythrocyte production or the response to bone marrow transplantation, 3) and bleeding [3].

Reticulocytes are immature forms of red blood cells (erythrocytes) that have just been released from the bone marrow into the bloodstream. The reticulocyte count reflects the activity of erythropoiesis (the production of red blood cells) in the bone marrow. An elevated reticulocyte level typically occurs in response to hemolytic anemia or blood loss, while a decreased count indicates impaired erythrocyte production [4].

Falciparum malaria is malaria caused by *P. falciparum* which is transmitted through by the bite of a female *Anopheles* mosquito. The clinical symptoms are irregular fever, anemia, splenomegaly, high parasitemia in the patients' blood and complications can occur [3]. The development of falciparum malaria is greater, if parasitemia is high then this parasite will attack of form of erythrocytes [3]. Falciparum malaria can cause prominent anemia with leucopenia and monocytosis. Fever caused by falciparum malaria occurs starting in the morning and decreases throughout the day.

Plasmodium falciparum, usually attacks young erythrocytes form such as reticulocytes to mature erythrocytes. This picture can be seen in peripheral blood examinations using thick and thin blood smears using an electric binocular microscope with 1000 X magnification and 1 to 2 drops of immersion oil and the parasite seen under the microscope. Is the young form of the parasite namely the ring form.[5].

Tertian malaria is a disease caused by *Plasmodium vivax* through the bite of the female *Anopheles* mosquito as a malaria vectors. The clinical symptom caused are afternoon and evening fever, chills, nausea, vomiting headache and dizziness, no desire to eat, and bitter mouth and tongue. *P. vivax* usually only infects erythrocytes as normoblasts and reticulocytes [6].

The difference in reticulocyte counts between patients infected with *Plasmodium falciparum*, which causes tropical malaria, and *P. vivax*, which causes tertian malaria, reflects the distinct erythrocyte tropism of each parasite species and the body's hematopoietic response to infection-induced anemia [7].

Plasmodium falciparum is capable of infecting red blood cells at all stages of maturation, including older erythrocytes. This broad range of invasion often leads to severe anemia due to extensive destruction of both infected and uninfected red blood cells. Additionally, *P. falciparum* infection can suppress erythropoiesis in the bone marrow, resulting in reduced reticulocyte production and contributing to the development of severe anemia. Other contributing factors include the presence of hemozoin (a malaria pigment), pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α), and oxidative stress, all of which play roles in inhibiting the maturation of erythroblasts into reticulocytes [7].

Plasmodium falciparum has the ability to infect erythrocytes at all stages of maturation, including aged red blood cells. This infection often leads to severe anemia due to extensive destruction of erythrocytes, both infected and uninfected. Moreover, *P. falciparum* can suppress erythropoiesis in the bone marrow, resulting in reduced production of reticulocytes, which further contributes to anemia. Additional factors that play a role in the development of severe anemia include the accumulation of hemozoin (a pigment produced by the malaria parasite), increased levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α), and oxidative stress. These elements collectively impair the maturation of erythroblasts into reticulocytes, thereby disrupting red blood cell regeneration [7].

Plasmodium vivax exhibits a strong preference for infecting reticulocytes, which are immature erythrocytes released from the bone marrow. This tropism triggers increased reticulocyte production as a compensatory response to hemolytic anemia. However, the reticulocytes infected by *P. vivax* are inherently less stable than erythrocytes infected by *P. falciparum*, leading to increased destruction of these immature red blood cells. This reduced stability may contribute to greater erythrocyte turnover and further exacerbate anemia [7].

Method and Study site

A total of 75 patients from Sentani community Health Center was infected through *Plasmodium falciparum* and *P. vivax*. After we were doing an informed consent. Malaria screening was used microscopy. The patients presented at the clinical laboratory with fever at Sentani Community Health Center. The diagnosis of malaria was the examination of thick and thin blood smears for malaria parasite by with Giemsa staining and finding parasite of *P. falciparum* and *P. vivax* with electric microscopy in clinical laboratory of Sentani Community Health Center, we used the standard procedure. After the detection of malarial parasites thick blood smears were used to identify parasite density of *P. falciparum* and *P. vivax* in thin blood smears were used to identify the parasites species of *Plasmodium*. [8,9]. To perform a reticulocytes count, we were using hemocytometer and Brilliant Cresyl Blue solution.

For malaria parasite density. The number of asexual parasites per μ l of thick and thin blood smears, will be calculated by dividing the number of parasites by the number of white blood cells counted and then multiplying by an assumed white blood cells density (8000 per μ l) [8,9].

The aim of this study was to determine the comparison of the

density of malaria parasites and counting the number of reticulocytes in patients suffering from falciparum malaria and vivax malaria who were treated at the Sentani Community Health Center.

Study Population and Ethical Clirence

This research was conducted at the Sentani Community Health Center, Jayapura District. Patients who came for treatment came from the cities of Sentani, Ifar besar, Yahim, Serehm Toladan and Kemiri. Sentani city has a rainy season that starts from January to April and from September to December. Average annual temperature each year. Patients were required to give written informed consent to this study which was duly explained to them in English and Indonesia. A questionnaire was administered to consented in order to obtain information on the demographic distribution of patients.

Detection of Malaria Parasite

Two thick and thin blood smears were prepared from each patients using both perpheral and venous. blood was obtained using finger prick and venous blood was obtained from blood drawn into EDTA tubes. The blood smears were air, -dried and stained with 3% Giemsa during 45-60 minutes [9-11].

To detection density of malaria parasite using a light electric microscopic, the result examination was obtained from a third blood smears was invited to confirm the result and the results obtained by third microscopist were presumed [8,9]. The thick were screened for 200 fields using the 100 x (with oil immersion)

objective. If density of malaria parasite were seen, the thin smear was then used to quantify parasitemia as well as were reached and the number determine the species of Plasmodium. The asexual stages of the Plasmodium were counted until 200 WBC were reached and the number obtained was divided by 200 and then multiplied by 8000 to give numbers in parasite per mm [8-10].

Diagnosis of Laboratory

Reticulocyte count

Reticulocytes are juvenile red cells; they contain remnants of the ribosomal ribonucleic acid (rRNA) that was present in larger amount in the cytoplasm of the nucleated precursors from which they were derived. Ribosomes have the property of reacting with certain basic dye such as azure B Brilliant Cresyl Blue to form a blue or purple prepi tate of granules. o filament [10-12].

Examination of Slide

Examination of Giemsa-stained blood smear using ligh microscopic is considered the gold standard of diagnosis [13-15]. Blood smears can be prepared using peripheral and venous blood with EDTA. The smears were also examined for staining characteristic of the smear as a whole and of malarial parasites of different stage species and the turnaround time and we were reporting in the result. In screening for density of malaria parasite, we used microscopic for evaluate each blood smears for its dilution staining pattern speed and case of reading the blood smears. The parasite density in the blood smears were calculate through counting the number of parasites per 200 white blood cells [16,17].

Result

Table 1: Data Of Count of Reticulocyte to Falciparum Malaria Patients Base on Parasite Density in Sentani Community Health Center

Parasitemia of P. falciparum	Reticulocyte		Parasite density (μL)	Frequency (%)	P value
	Low	Increase			
Positive (+1)	20	0	40 -400	20(50)	0.000
Positive (+2)	2	10	401-4000	12 (30)	
Positive (+3)	0	6	4001-39.999	6 (15)	
Positive (+4)	0	2	≥ 40.000	2 (5)	
Total	22	18	-	40 (100)	

The results presented in Table 1 show that increased reticulocyte counts were observed in patients with tropical malaria at a parasite density of 401–4,000/μL in 10 (25%) patients, at 4,001–39,999/μL in 6 (15%) patients, and at ≥ 40,000/μL in 2 (5%) patients. Based on the Chi-Square statistical test, the ob-

tained p-value was 0.000 (< 0.05), with a 95% confidence interval (CI). Therefore, there is a statistically significant association between parasite density and reticulocyte count in patients with falciparum malaria.

Table 2: Data of Count of Reticulocyte to Tertian Malaria patients base on parasite density In Sentani Community Health Center

Parasitemia of. P.vivax	Reticulocyte		Parasite density (μL)	Frequency (%)	P.value
	Low	Increase			
Positive (+1)	8	4	40 -400	4	0.233
Positive (+2)	3	6	401-4000	6	
Positive (+3)	3	5	4001-39.999	5	
Positive (+4)	2	5	≥40.000	5	
Total	16	19		35	

The results of the study indicated that elevated reticulocyte counts were observed in patients with tertian malaria at a parasite density of 401–4,000/ μ L in 4 patients (11.4%), at 4,001–39,999/ μ L in 5 (14.3%) patients, and at $\geq 40,000$ / μ L in 5 (14.3%) patients. Based on the Chi-Square statistical test, the p-value was 0.233 (> 0.05) with a 95% confidence interval (CI), indicating that there is no statistically significant association between parasite density and reticulocyte count in patients with tertian malaria.

Discussions

The findings presented in Table 1 indicate that lower reticulocyte counts were observed in patients with tropical malaria at a parasite density of 401–4,000/ μ L, whereas higher reticulocyte counts were found in patients with *Plasmodium falciparum* malaria at parasite densities of 4,001–39,999/ μ L and $\geq 40,000$ / μ L. In tropical malaria infections, particularly those caused by *P. falciparum*, reticulocyte levels in the blood tend to decrease during the acute phase of infection, despite the presence of anemia. This reduction is attributed to suppressed erythropoiesis in the bone marrow due to the toxic and immunological effects of the infection. The main mechanisms involved include the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α) and interferon-gamma (IFN- γ), which directly inhibit the differentiation and proliferation of erythroid precursor cells. Additionally, the presence of hemozoin a pigment derived from hemoglobin metabolism by malaria parasites also plays a role in suppressing bone marrow activity by affecting the hematopoietic micro environment. A study by demonstrated that hemozoin accumulation in macrophages correlates with reduced reticulocyte production both in vitro and in vivo [18,19].

An increased reticulocyte counts in patients with tropical malaria at parasite densities of 4,001–39,999/ μ L and $\geq 40,000$ / μ L can be interpreted as a physiological response to anemia caused by *Plasmodium falciparum* infection. At this stage, although *P. falciparum* continues to cause hemolysis of both infected and uninfected erythrocytes, bone marrow activity is not yet fully suppressed, as seen in severe infections. Therefore, the bone marrow is still capable of maintaining—or even increasing—erythropoiesis as a compensatory mechanism in response to erythrocyte loss [20]. demonstrated that at moderate levels of parasitemia, there is activation of erythropoiesis indicated by elevated reticulocyte counts, in contrast to high parasitemia, which shows a significant decline due to impaired hematopoiesis [1]. Additionally, observed mild to moderate reticulocytosis in malaria patients with moderate anemia, suggesting that the bone marrow's compensatory capacity remains functional during this phase (2,21). Thus, the increase in reticulocytes in patients with parasitemia of 4,001–39,999/ μ L and $\geq 40,000$ / μ L can be understood as a normal physiological response to moderate hemolytic anemia, as long as bone marrow suppression due to inflammatory or toxic effects of the parasite has not yet occurred.

The results in Table 2 indicate that patients with tertian malaria caused by *Plasmodium vivax* and moderate levels of parasitemia (approximately 4,001–39,999/ μ L and $\geq 40,000$ / μ L) generally exhibit mild to moderate increases in reticulocyte counts. This finding is closely related to the physiological response of the bone marrow to hemolytic anemia caused by malaria infection. *P. vivax* has a strong preference for infecting young erythrocytes (reticulocytes). As a result, although infection may lead to hemo-

lysis and anemia, the degree of bone marrow suppression is less severe than that caused by *P. falciparum*, thereby allowing compensatory erythropoietic activity to occur. A study by reported that patients with *P. vivax* malaria exhibited increased reticulocyte counts as a response to mild to moderate hemolytic anemia, typically associated with moderate parasitemia levels [1,22]. Similarly, confirmed that while *P. vivax* can cause significant anemia, bone marrow regenerative activity is often preserved, as indicated by increased reticulocyte production [2,23]. Clinically, reticulocytosis in tertian malaria with moderate parasitemia (4,001–39,999/ μ L and $\geq 40,000$ / μ L) suggests that hematopoietic function remains intact, and the body is actively compensating for erythrocyte loss due to hemolysis. Laboratory findings typically show an elevated reticulocyte percentage (e.g., $>2\%$), depending on the severity of anemia and the individual's physiological response to infe.

Conclusion

This study shows that in *falciparum* malaria, reticulocyte count increases were found starting from parasitemia levels +2 to +4. In contrast in patients infected with *P. vivax*, increased reticulocyte counts were observed across a wider range of parasitemia levels, from +1 to +4. These findings indicate a differing pattern of bone marrow response to parasitic infection in the two types of malaria.

In *P. vivax* infection, because of stimulation of erythropoiesis occurs due to the loss of young red blood cells; therefore, the body will sustain the production of new erythrocytes.

In *P. falciparum* infection, the reticulocyte count can be markedly elevated because all erythrocyte stages can be infected; however, the peak increase in reticulocytes is usually observed after moderate to severe parasitemia (positive 2–4) and once the suppression of erythropoiesis has diminished.

Author's Contribution

Field work was done and Laboratory work was conducted at Sentani Community Health Center at Sentani district.Papua Province. The Author participated in the design of the study, the analysis and the interpretation of the result, as well as in preparation the manuscript.

Acknowledgments

We would like to express to our sincere gratitude to the Head of the Sentani City Public Health Center and to our colleagues at the Sentani City Public health Center Laboratory. We participated as authors in designing this study, analyzing and interpreting the research finding, and the manuscript for publication.

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