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Association of Parasite Density and Hematological Parameters of *Plasmodium vivax*- and *Plasmodium falciparum*-infected Patients Attending Merauke General Hospital, Papua, Indonesia

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Abstract

BACKGROUND: *Plasmodium falciparum* and *Plasmodium vivax* are frequent causes of malaria. Although they are blood parasites, their biological characteristics are dissimilar, and their species-related consequences on hematological parameters have not been widely investigated. They might be valuable to distinguish both species infection, notably for an endemic region with limited diagnostic resources.

AIM: This study aimed to know the species-specific effect on hematological parameters and its correlation to the parasite density in *P. vivax*- and *P. falciparum*-infected patients attending Merauke General Hospital, Papua, Indonesia.

MATERIALS AND METHODS: Malaria patients confirmed by blood film microscopy from January 1 to July 31, 2019, were recruited, and their hematological parameters were measured using Sysmex XN-1000 instrument. All obtained data were analyzed statistically.

RESULTS: From 100 malaria-positive patients, 87 patients, consisting of 57 *P. vivax* and 30 *P. falciparum* patients, met criteria. Anemia and parasite density >50,000 parasites/μL were significantly higher in *P. falciparum* than *P. vivax* patients ($p < 0.05$) though hemoglobin concentration and parasite density were insignificantly different. Interestingly, basophil count was significantly higher in *P. falciparum* compared to *P. vivax* patients ($p = 0.04$). The eosinophil count was significantly higher in *P. vivax* ($p = 0.01$) than *P. falciparum* patients and indicated a significant positive correlation ($p = 0.04$, $r = +0.28$) with the parasite density.

CONCLUSION: There were significant differences between basophil and eosinophil count between *P. vivax* and *P. falciparum* infections. Eosinophil count showed a significant positive correlation with parasite density.

Introduction

Malaria remains one of the deadly infectious diseases that continue as serious public health problems worldwide, particularly in tropical countries. Around 219 million peoples suffered from malaria, and 445 thousand of them died [1]. Indonesia, the country with a high malaria burden, has committed to eradicating malaria in 2030 [2]. Instead, the latest report shows the increased cases of more than 100,000 malaria cases occurred between 2017 and 2016 in Indonesia [1].

Dynamic alterations of hematological parameters are common findings in malaria patients. They might attribute to some changes in significant blood cell types, including erythrocyte, leukocyte, and platelet [3], [4], [5], [6]. Hemoglobin, erythrocyte, total leukocyte, neutrophil, eosinophil, monocyte, and lymphocyte counts seemed significantly lower contrasted with non-malaria patients [3], [4]. Meanwhile, erythrocyte indices, neutrophil-lymphocyte ratio (NLR), and monocyte-lymphocyte ratio (MLR) were higher opposed to non-malaria patients [5], [7]. Leukocyte, neutrophil, monocyte, lymphocyte, erythrocyte, and platelet counts among different levels of parasitemia levels also marked to differ significantly [5].

Plasmodium falciparum and *Plasmodium vivax* are frequent causes of malaria worldwide, including in Indonesia [1]. Although both species are blood parasites, their biological characteristics, such as life cycle, virulence mechanisms, disease manifestation, and management, are dissimilar [8]. Their species-related consequences

on hematological parameters have not been widely investigated. For instance, *P. falciparum* might result in more than 10% parasitemia, whereas *P. vivax* could only reach 2% [8]. Higher parasitemia would undoubtedly result in poor prognosis [9], [10]. However, severe *P. vivax* malaria cases have also been recorded [6], [11]. That evidence inferred that prompt species identification is necessary since both infections require different pharmacotherapeutic approaches. Unfortunately, the microscopic blood smear, the gold standard for diagnosing malaria, needs more extended time, and particular expertise to obtain the result [12]. Hematological parameter changes associated with parasite species-specific effects appear potential to provide a reference in specifically suspecting malaria species, even for estimating the total parasite burden of the disease in the endemic area, due to its availability in the routine examination. The present study aimed to investigate the comparison of hematological parameters between *P. vivax*- and *P. falciparum*-infected patients referred to Merauke General Hospital. The study also tried to correlate the parasite density and hematological parameters in each investigated group. Better understanding in this field could help physicians suspect parasite species in highly suspected malaria patients and predict patient prognosis so that the quick and specific management could be initiated, particularly in the endemic area with limited diagnostic facilities.

Materials and Methods

Samples collection

A cross-sectional study was conducted at Merauke General Hospital, Papua, Indonesia, from January to July 2019. Blood samples were collected from patients by vena punctured after signing the informed consent. Blood, then, was transferred to EDTA tubes for parasite and hematology examination. Patients with microscopically confirmed malaria of *P. vivax* or *P. falciparum* were engaged, whereas those with malaria mixed infection, immunocompromised, and comorbid infections were excluded.

Malaria parasite examination

Microscopic examinations were carried out on prepared Giemsa-stained thin and thick blood films to identify malaria species and quantify the parasite density based on the latest WHO guidelines [13]. The formula employed to quantify parasite density is written below.

$$\text{Parasite density}\left(\frac{\text{parasites}}{\mu\text{L}}\right) = \frac{\sum \text{counted parasites}}{200 \text{ observed leukocytes}} \times \text{leukocyte count}$$

The examination was accomplished by two trained and experienced field microscopists independently in Merauke clinical pathology laboratory. All obtained data were reassessed by the clinical pathologist at Merauke General Hospital.

Hematology assay

The hematology examination was performed using *Sysmex* XN-1000 (*Sysmex* Corporation, Kobe, Japan). The examination included hemoglobin concentration, erythrocyte count, mean corpuscular volume, mean corpuscular hemoglobin (MCH), MCH concentration (MCHC), leukocyte and differential counts, NLR, MLR, and platelet count. All laboratory procedures were conducted based on appropriate standard operating procedures. Quality control of the instrument was completed per manufacturer standards. As parasite examination, all obtained data were reassessed by the clinical pathologist at Merauke General Hospital.

Data analysis

Normality distribution of data was analyzed using the Kolmogorov–Smirnov test if samples exceed 50 and the Shapiro–Wilk if samples were 50 or lesser. Our comparison hypothesis was proved by performing the independent t-test for normally distributed data. Otherwise, the Mann–Whitney U-test would be used. The proportion of data was analyzed using the Z test. The Pearson correlation test was employed to test our correlation hypothesis if data were normally distributed, and the Spearman correlation test in case they did not. The finding was deemed significant at $p < 0.05$ or $|Z| > 1.65$.

Ethical declaration

This study's ethical clearance was issued by the Medical Research Ethics Committee, Faculty of Medicine, Universitas Airlangga (No.169/EC/KEPK/FKUA/2019).

Results

General characteristics

A total of 100 positive malaria samples were collected. However, six patients with mixed infection (*P. vivax*/ *P. falciparum*) and seven patients with incomplete data were excluded. The study eventually involved 57 (65.51%) cases of *P. vivax* and 30 (34.48%) cases of *P. falciparum* infections. As many as, 64.37% of involved patients were male and

became the predominant contributor of malaria cases, which comprised 64.91% of *P. vivax* and 63.33% of *P. falciparum* cases. The mean age of *P. vivax* and *P. falciparum* groups was 29.87 ± 16.03 and 29.08 ± 15.33, respectively.

Comparison of parasite density between P. vivax and P. falciparum infection

The median of *P. vivax* and *P. falciparum* parasite density was 5902.04 (379.84–135,700.00) parasites/μL and 9061.00 (54.48–1109.675) parasites/μL, respectively. The median between both species was insignificantly different (p > 0.05). Furthermore, parasite density data were grouped into three categories, as shown in Table 1, and noted for their proportion in each malaria species to be compared statistically. The proportion of the parasite density >50,000 parasites/μL was significantly higher in *P. falciparum* compared to *P. vivax* (|Z| = 1.87, p = 0.03).

Table 1: Parasite density between *Plasmodium vivax* and *P. falciparum*

Parasite density (parasites/μL)	Number (%)				*Z*-score	p-value
	P. vivax		*P. falciparum*		(95% CI)	
Category 1 (<10,000)	37	(64.91)	16	(53.33)	1.05	0.15
Category 2 (10,000–50,000)	15	(26.32)	7	(23.33)	0.30	0.38
Category 3 (>50,000)	5	(8.77)	7	(23.33)	−1.87	0.03[a]
Total	57	(100.00)	30	(100.00)	N/A	N/A

[a]Significant value at |Z| >1.65, equal to *p* 0.05 (one tailed) N/A: Not applicable. *P. falciparum: Plasmodium falciparum, P. vivax: Plasmodium vivax.*

Comparison of hematological parameters between P. vivax and P. falciparum infection

This study found that both eosinophil and basophil count were significantly different between investigated groups. *P. vivax* group's basophil count was substantially lower compared to *P. falciparum* group (p = 0.04). Still, *P. vivax* group's eosinophil count was significantly higher contrasted to *P. falciparum* group (p = 0.01) (Table 2). Nonetheless, hemoglobin concentration, erythrocyte indices, erythrocyte count, total leukocyte count, neutrophil count, lymphocyte count, monocyte count, platelet count, NLR, and MLR between both groups were considered insignificantly different (p > 0.05) (Table 2).

Table 2: Comparison of hematological parameters between *P. vivax* and *P. falciparum* infections

Parameters	Mean ± SD/Median (Min.–Max.)				p-value
	P. vivax		*P. falciparum*		
Hemoglobin (g/dL)	12.60	(4.30–16.60)	11.95	± 2.78	0.29
Erythrocyte (×10^6/μl)	4.81	± 0.76	4.56	± 0.97	0.19[b]
MCV (fL)	74.93	± 8.88	75.82	± 8.26	0.65[b]
MCH (pg/cell)	26.70	(14.50–32.90)	25.50	(21.30 – 46.10)	0.82
MCHC (g/dL)	34.60	(25.80–38.40)	34.65	(31.20 – 46.30)	0.72
Platelet (×10^3/μL)	97.00	(24.00–481.00)	92.00	(13.00 – 285.00)	0.19
Leukocyte (×10^3/μL)	7.10	(3.14–17.03)	6.48	(2.19 – 19.66)	0.92
Eosinophil (×10^3/μL)	0.10	(0.00–3.48)	0.05	(0.00 – 0.60)	0.01[c]
Basophil (×10^3/μL)	0.02	(0.00–0.11)	0.03	(0.00 – 0.20)	0.04[c]
Neutrophil (×10^3/μL)	5.04	(2.12–14.00)	5.69	± 2.83	0.71
Lymphocyte (×10^3/μL)	1.05	(0.18–3.13)	1.15	(0.33 – 4.05)	0.20
Monocyte (×10^3/μL)	0.62	± 0.40	0.57	(0.16 – 2.20)	0.63
NLR	4.95	(1.40–18.27)	4.75	(0.17 – 24.18)	0.28
MLR	0.55	±0.29	0.47	(0.21 – 1.13)	0.66

P. falciparum: *Plasmodium falciparum, P. vivax: Plasmodium vivax, MCV*: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, NLR: Neutrophil-lymphocyte ratio, MLR: Monocyte-lymphocyte ratio.

Furthermore, the occurrence of anemia was significantly different between both species. Anemia conduced to occur in *P. falciparum* group contrasted to *P. vivax* group (|Z| = 2.48, p = 0.01) (Table 3). Nevertheless, the occurrence of leukocytosis, leukocytopenia, thrombocytosis, and thrombocytopenia was different insignificantly.

Table 3: Major hematological abnormalities in *P. vivax* and *P. falciparum* infection

Major hematological abnormalities	Number (%)				*Z*-score (95% CI)	p-value
	P. vivax		*P. falciparum*			
Anemia (Hb ≤11 g/dL)	12	(21.05)	14	(46.67)	−2.48	0.01[a]
Leukocytosis (>10,000 cells/μL)	7	(12.3)	6	(20.00)	−0.96	0.17
Leukopenia (<4000 cells/μL)	4	(7.00)	1	(3.00)	0.70	0.24
Thrombocytosis (>450,000 cells/μL)	1	(1.75)	0	(0.00)	0.73	0.23
Thrombocytopenia (<150,000 cells/μL)	41	(71.93)	24	(80.00)	−0.82	0.21

Hb: Hemoglobin, [a]significant value at |Z| > 1.65, equal to p < 0.05 (one tailed). *P. falciparum: Plasmodium falciparum, P. vivax: Plasmodium vivax.*

Correlation between parasite density and hematological parameters

The parasite density and eosinophil count in *P. vivax* group indicated a significant positive correlation with r = +0.28 (p = 0.04) (Table 2). Hemoglobin concentration, erythrocyte count and indices, platelet count, lymphocyte count, total leukocyte count, basophil count, neutrophil count, monocyte count, NLR, and MLR showed insignificant correlation. However, in *P. falciparum* group, neither parasite density nor hematological parameters seemed to correlate significantly.

Discussion

Hematological abnormalities become a prevailing hallmark during malaria and favor to be variable among studies. These diversities appear to be multifactorial in etiology, depending on the parasitic agent, host responses, and environment. *P. vivax* and *P. falciparum* are prevalent causes of malaria in Indonesia [1], while both species possess different characteristics, pathogenesis mechanisms, and therapeutic management [8]. Although the life cycle, biological aspects, and clinical manifestations between both parasites have been studied extensively, the parasite density might differ among endemic areas, and hematological differences resulting from different malaria species seem to lack evidence. This research was conducted based on issues that those species distinctiveness might result in parasite density and hematological parameter abnormalities, which probably own clinical significance in the endemic area. To the best of our knowledge, this was the first study comparing various hematological parameters of *P. vivax* and *P. falciparum* patients and correlating those parameters with the malaria parasite density in Indonesia, particularly in Papua.

The primary cause of malaria in the Merauke was *P. vivax* followed by *P. falciparum*. Six patients with mixed infection (*P. vivax* and *P. falciparum*) were unexpectedly observed. This result contrasts with the study in Jayapura, Papua, which was predominantly caused by *P. falciparum* [14]. Those males were likely to get malaria than females, and insignificant patients' age difference satisfied previous findings [3], [5] and seemed to associate with different occupational exposures.

High parasitemia is likely to occur in *P. falciparum* compared to *P. vivax* due to the parasite indiscrimination to infect all erythrocyte maturation stages, generate multiple infections, and perform rapid schizogony [8]. This study showed that the proportion of parasite density >50,000 parasites/μL in *P. falciparum* was significantly higher as opposed to *P. vivax* infection (|Z| = 1.87, p = 0.03) (Table 1). This study's highest parasite density was 135,700 parasites/μL for *P. vivax* and 1109.675 parasites/μL for *P. falciparum*. This finding of parasite propensity concords the previous study reported by Barber *et al*. [10]. Nevertheless, the median of parasite density between both groups was not statistically different. This finding might happen since the evidence of *P. falciparum*-infected erythrocyte performing cytoadherence have been reported [15], [16], resulting in parasite sequestration and preventing circulating parasite to represent the total parasite biomass. Notwithstanding, the present study still confirms the tendency and ability of *P. falciparum* to result in very high parasitemia.

As parasite density relates to the severity of malaria infection [9], [10], it is necessary to correlate the parasite density in each species with common examined hematological parameters. Although, none variables posed a significant correlation, except for eosinophil count in *P. vivax* infection. The parasite density showed significant positive correlation with eosinophil count (r = 0.28, p = 0.04) (Table 4). These results appear contrary to some studies that indicated several significant correlations. An inverse correlation between the parasite densities with the lymphocyte count in *P. falciparum* infection and positive correlations of the parasite density with neutrophil and platelet counts in *P. vivax* and *P. falciparum* infection were reported by studies in Ghana and South Ethiopia [3], [7]. A mathematical model study also designated good relationships of the parasite density with neutrophil, lymphocyte, monocyte, and eosinophil count in *P. falciparum* infection [17]. Despite those discordances, insignificant correlations of parasite density, in both species, with hemoglobin concentration, leukocyte count, and monocyte count, in this study, agree with the previous studies [3], [7]. Interestingly, the positive correlation of eosinophil count and parasite density in *P. vivax* group seems becoming a new finding referring to those studies.

Table 4: Correlation between parasite density and hematological parameters in *P. vivax* and *P. falciparum* infection

Parameters	*P. vivax*		*P. falciparum*	
	Correlation coefficient	p-value[a]	Correlation coefficient	p-value[a]
Parasite density (parasite/μL)				
Hemoglobin	−0.20	0.15	0.13	0.50
Erythrocyte	–0.13	0.33	0.07	0.71
MCV	–0.11	0.43	0.14	0.47
MCH	–0.16	0.25	0.18	0.35
MCHC	–0.15	0.26	0.24	0.20
Platelet	–0.13	0.34	–0.08	0.68
Leukocyte	0.14	0.32	0.16	0.40
Eosinophil	0.28	0.04[b]	–0.16	0.39
Basophil	0.20	0.14	–0.11	0.56
Neutrophil	0.07	0.59	0.26	0.17
Lymphocyte	–0.04	0.76	–0.17	0.37
Monocyte	0.13	0.34	–0.13	0.51
NLR	0.13	0.34	0.29	0.12
MLR	0.18	0.18	–0.05	0.78

[a]p-value by Spearman's rho correlation test, [b]significant value at p < 0.05. *P. falciparum: Plasmodium falciparum, P. vivax: Plasmodium vivax, MCV:* Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, NLR: Neutrophil-lymphocyte ratio, MLR: Monocyte-lymphocyte ratio.

Interplays of parasite and hematological parameters still require further elucidation. Intriguing reasons that might underlie insignificant correlations influence malaria transmission, sequestration, and systemic inflammatory reactions [15], [18], [19]. Pro-inflammatory cytokines generated during *P. falciparum* infection promote endothelial activation, leading to leukocyte adherence and sequestration, microvascular thrombosis, and endothelial injury (increased platelet usage) [10], [19], [20], [21], [22]. They might explain why the significant positive correlation of eosinophil and the parasite density, in this study, only occurred in *P. vivax* infection since severe inflammatory reactions during *P. vivax* infection are unlikely to occur [8], [20]. However, the reasons for eosinophil roles during malaria are still controversial. They have been associated with an antiparasitic activity, disease recovery, hypersensitivity, and total parasite burdens [23], [24], [25], [26].

Anemia is frequently observed and associated with malaria severity. However, its exact mechanism seems intricate and poorly understood. As the parasites invade erythrocytes, hemolysis will be occurring and lead to anemia. Nonetheless, the loss of uninfected erythrocytes (UE) appears more influential in the anemia pathogenesis. The previous studies inferred that either mechanical or immune-mediated splenic clearance was principal underlying causes of massive UE loss [27], [28]. Bone marrow suppression was also responsible for the anemia occurrence [29], [30]. The anemia (hemoglobin <11 g/dL) in both *P. vivax* and *P. falciparum* was noted in our study. It favored in *P. falciparum* compared to *P. vivax* (|Z| = 2.48, p = 0.01) (Table 3). Erythrocyte profiles in *P. falciparum*, however, did not significantly differ from *P. vivax* (Table 2). This result argues the earlier study that found significant differences in erythrocyte count and indices, even if it strengthens the finding of the insignificant hemoglobin concentration difference in both species [5], [11]. These results possibly happen as different research settings might possess different local parasitic strains and malaria transmission intensity. Evidence of *P. falciparum*-infected erythrocyte sequestration [15], [16] makes measured circulating erythrocyte profiles unable to figure out actual states.

The leukocyte deems as the significant element of the immune system that deals with the plasmodia invasion. It prefers to change, meeting on needs and agent properties dynamically. Theoretically, massive schizont ruptures, particularly in *P. falciparum*, will be followed by the extensive exposure with intraerythrocytic materials and parasite antigens, driving to profound immune responses [31]. Our finding has met the theory that leukocytosis (leukocyte >10,000 cells/μL) favored occurring in *P. falciparum* rather than *P. vivax* although it was statistically insignificant. That leukocytopenia (leukocyte <4000 cells/μL) was found more in *P. vivax* (Table 3) which was in contrast with a report in Thailand during 1998–1999 [32]. Furthermore, leukocyte profiles, such as total leukocyte count, neutrophil count, lymphocyte count, monocyte count, NLR, and MLR, noted insignificant differences between groups. Nevertheless, the significant difference of eosinophil count ($p = 0.01$) and insignificant differences of total leukocyte, lymphocyte, and monocyte counts still followed an investigation in Thailand [5]. That basophil count marked to be significantly different ($p = 0.04$) between observed groups may become a new finding referring to that study. Unimportant differences of NLR and MLR between both species were found, although the previous result showed significant differences compared to non-malaria patients [4].

Inconsistent findings of leukocyte profiles between investigated groups are not unlikely. Microvascular endothelial activation, induced by increased pro-inflammatory cytokines, such as tumor necrosis factor-α and interleukin (IL)-1β, leads to overexpression of intercellular adhesion molecule-1 and secretion of IL-8, a potent neutrophil attractant [31], [33]. They promote leukocytes marginalization and sequestration [21], resulting in discrepancies between circulating and actual total leukocyte count. That the basophil count appeared higher in *P. falciparum* than in *P. vivax* patients may support the finding of *P. falciparum* ability to produce histamine-releasing factors, belonging to translationally controlled tumor protein (TCTP), suggesting important roles in regulating basophil and eosinophil activity [26], [34]. A study in Senegal reported a higher concentration of *P. falciparum* TCTP in patients with severe malaria than mild malaria and healthy control [35]. That study also identified the enhanced basophil reactivity to stimuli associated with *P. falciparum* TCTP *in vivo* and with malaria severity [36]. TCTP might also reflect total parasite burdens (including sequestered parasite) rather than peripheral parasitemia [26]. This mechanism explains why both parasite densities in our research do not exhibit significant differences regarding the discovery of a considerable difference in eosinophil and basophil count in investigated species. However, the eosinophil count was higher in *P. vivax* than *P. falciparum* might indicate other different *P. vivax* pathogenesis processes that need further elucidation.

Another common complication of malaria is thrombocytopenia. Its pathogenesis has not been certainly ascertained. Platelet-mediated erythrocyte clumping, von Willebrand factor adherence, sequestration, and immune-mediated destruction are responsible for thrombocytopenic episodes [22], [35], [37], [38], [39]. That thrombocytopenia (<150,000 cells/μL) was still a common finding was confirmed in this study (>70% of patients in both groups), and its proportion of both species was roughly similar (Table 3), as reported in another study [4]. A thrombocytosis case was only discovered in *P. vivax* group. Nonetheless, the comparison of platelet count between investigated species was not significantly different, in contrast with the previous investigation in Thailand [5].

This study's limitation was no assessment of primary confounding factors affecting hematological parameters such as the engaged patient's genetic susceptibilities and micronutrient state. That the parasite examination only relied on microscopy also makes submicroscopic malaria unrecognized and could lead to underestimation of parasite density impact on hematological parameters.

Conclusion

Diversities of hematological abnormalities during malaria are common findings. Anemia, leukocyte count abnormalities, and thrombocytopenia are predominant and favor to be in *P. falciparum* infection. Several parameters do not differ significantly between *P. vivax* and *P. falciparum.* However, basophil count is significantly higher in *P. falciparum* infection, whereas eosinophil count is significantly higher in *P. vivax* infection. These findings suggest that basophil and eosinophil count possesses clinical values and might provide a clue, together with clinical presentations, to suspect *P. vivax* or *P. falciparum* infection in the endemic area. The significant positive correlation between the parasite density and eosinophil count in *P. vivax* may be the potential to predict the total parasite burden. These findings offer the opportunity to conduct prompt and specific management, particularly in regions with limited diagnostic facilities. Furthermore, since this report is a pioneer study in Merauke, Papua, researches concerning this topic should be more encouraged.

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