ORIGINAL ARTICLE

Relationship of Malaria Infection (*Plasmodium Falciparum & Plasmodium Vivax*) to Average Haemoglobin (Hb) Levels in Malaria Endemic Areas of Eastern Indonesia

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ABSTRACT

Introduction: The prevalence of malaria infection in Eastern Indonesia remains high (Annual Parasite Incidence/API > 5%). East Nusa Tenggara (ENT) province is one of the endemic regions that lacks data comparing haemoglobin (Hb) levels between *P. falciparum* and *P. vivax* infection. Therefore, this study aimed to assess the relationship between malaria infection and Hb levels in different level of API areas of endemicity in Eastern Indonesia. **Materials and methods:** A cross-sectional study was conducted in Eastern Indonesia. Samples were selected by systematic random sampling from primary health care records. Hb levels were measured using point-of-care haemoglobin testing with capillary blood by local health practitioners. Malaria species were examined by microscopy and nested Polymerase Chain Reaction (PCR). Other variables, including gender, age, residence, occupation, duration in endemic areas, and BMI were assessed. Data analysis was conducted using univariate, bivariate, multivariate, and t-test with 95% confidence interval (CI) and α=0.05. **Results:** A total of 510 respondents were included in this study. Malaria infection was detected in 148 respondents (29.1%), with 92 (62.1%) infected with *P. vivax* and 56 (37.8%) with P. falciparum. Anaemia was present in 278 respondents (54.5%), primarily among females (156 respondents). A significant correlation was observed between malaria infection and reduced Hb levels ($p<0.05$). **Conclusion:** Malaria infection was associated with decrease in Hb levels. There was no significant difference in Hb levels between *P. falciparum* and *P. vivax* malaria infections.
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INTRODUCTION

Malaria is an infectious disease caused by *Plasmodium* through the bite of a female *Anopheles* mosquito and will multiply in human red blood cells (RBC) (1). If left untreated, the disease may result in severe complications and eventually lead to death. According to the Indonesia Health Profiles 2018, provinces in Eastern Indonesia reported high annual parasite incidence (API) and showed varying prevalence levels of malaria cases: Papua Province with 52.99 per 1,000 residents, West Papua Province with an API of 8.49 per 1,000 residents, and East Nusa Tenggara Province with an API of 3.42 per 1,000 residents. API represents the total confirmed cases per thousand population. These API levels were still far from achieving malaria elimination compared to the national API (2).

Indonesia still utilizes microscopy with Giemsa 3% and rapid diagnostic test (RDT) as standard diagnostic examination. The accuracy of malaria diagnoses by microscopy depends on the level of competency, particularly in remote areas, due to challenges in producing high-quality malaria blood smears and variations in experience (3). Additionally, the sensitivity of RDT for detecting *P. falciparum* and *P. vivax* is lower compared to the polymerase chain reaction (PCR)

method (4).

Most malarial infections are associated with some degree of anaemia; however, the severity depends on patient-specific characteristics such as age, innate and acquired immunity, comorbidities, and parasite-specific factors. In regions with high malaria transmission rates, severe anaemia is a common complication associated with both falciparum and vivax malaria (5). Anaemia in malaria is caused by the destruction of erythrocytes by parasites when the schizont ruptures, accelerated destruction of non-infected erythrocytes, and the disintegration of erythrocytes. The greatest contributing factor to anaemia in malaria is the destruction of non-infected erythrocytes. In infected individuals, there is an increase in the clearance capacity and a decrease in the spleen's threshold for the clearance of abnormal erythrocytes, leading to a shortened erythrocyte lifespan (6).

A study by Douglas et al. in Southern Papua, involving a sample size of 922,120 patients, revealed a reduction in haemoglobin concentration associated with malaria. Mixed *Plasmodium* infection was associated with the highest risk of severe anaemia compared to *P. falciparum* and *P. vivax*. In patients with *P. falciparum* infection, the erythrocyte count and haemoglobin concentration were significantly lower than in patients with infections with reticulocytes *P. vivax* (7).

Risk factors for anaemia include the type of *Plasmodium* species, immunity status of the host, the presence of autoimmune hemolysis, sequestration of both infected and normal erythrocytes by the spleen, and disorders related to erythropoiesis (8). A study by Wirth et al. concluded that malaria was the most consistent risk factor for anaemia in children and non-pregnant women, alongside acute and chronic inflammation (9). Malaria is linked to iron deficiency, nutritional deficiencies, hookworm, bacterial infections, and haemoglobinopathies. Furthermore, another study by Sakwe et al. in low-income communities highlighted a significant link between anaemia and malnutrition, with malnutrition being strongly associated with malaria status (10).

Unfortunately, research on the relationship between Hb and malaria infection in Eastern Indonesia is limited, especially in regions with high API levels. This study aimed to assess the relationship between malaria infection and Hb levels in different level API areas of endemicity in Eastern Indonesia.

MATERIALS AND METHODS

Study location

This study was a cross-sectional study conducted in five areas (sub-districts) with different API levels in Eastern Indonesia from March 2019 to June 2020. South Central Timor (SCT) Regency Eastern Indonesia consists of 32 subdistricts and 278 villages covering an area of about 3,955.36 km^2 with a total of 468,346 residents (11). Agriculture is the primary sector of the economy. Approximately 27.84% of the SCT population are still living in poverty. SCT's health facilities include two hospitals, four polyclinics, 35 primary health care (PHC), and 37 subsidiary PHC. Between 2016 and 2018, there were 14,382 recorded cases of malaria (12).

Based on the data of API levels from the Health Department of SCT Regency, three areas included in this study were South Amanatun and Central Amanuban (API > 5 ‰), South Amanuban and Batu Putih (API 1 - ≤ 5‰), and Oenino (API ≤ 1‰) (11).

Sample size

A total of 510 respondents were selected by systematic random sampling based on the data of household membership documents from each selected area. The sample size was determined using the formula $n = z^2pq/d^2$, where n = the sample size required, z = 1.96: confidence level test statistic at the desired level of significance, p = 26% proportion of malaria prevalence, q = 1-p: proportion of malaria negative and d = 0,05: acceptable error willing to be committed. Based on the mentioned formula, the minimum sample size was 296. This was adjusted to an optimum of at least 510 samples. The inclusion criteria were individuals who had fever of less than a week and those above 5 years old, while those who were unwilling to participate in this study were excluded.

Data Collection

Each respondent was given information about the study and an informed consent was obtained from each respondent. Questionnaires were used to collect information regarding sociodemographic data (gender, age, residence, occupation, and length of stay (years) in endemic areas). The weight and height of each respondent were measured to determine the body mass index (BMI). BMI was classified based on the National Institute of Health (NIH) and WHO guidelines: a BMI <18.5 kg/m^2 was considered underweight, 18.5-24.9 kg/m^2 as normal weight, and ≥25 kg/m^2 as overweight or obese (13).

Hb levels were measured on-site by local health practitioners using standardised and calibrated devices (HB Bene-check stick test). The instrument measured Hb levels in capillary whole blood from the fingertip with a sample volume of 1µL and an accuracy of ≥ 95% (14). Hb levels were categorised according to WHO criteria to identify respondents with anaemia, with males having Hb levels < 13 g/dL and < 12 g/dL in females (15). Blood samples of approximately 5 mL were collected from each respondent and placed in EDTA tubes for *Plasmodium* genes analysis. Additionally, a finger-prick blood sample was taken for thick and thin blood films, which were

examined at the Malaria District Health Laboratory SCT and Parasitology Laboratory Faculty of Medicine, Public Health and Nursing Gadjah Mada University.

Nested PCR was carried out to determine the malaria species in The Laboratory for Research and Development of Vectors and Disease Reservoir, Ministry of Health Research and Development in Salatiga, using the Go Tag Green Master Mix, Promega, Cat No: M-7122, Madison, USA. DNA was isolated from whole blood samples collected in EDTA tubes. In the first round of nested PCR, *Plasmodium* genes were amplified using forward (F) primer r-PLU-5 and reverse (R) primer r-PLU-6. The second round of nested PCR targeted *Plasmodium* species (*P. falciparum* and *P. vivax*) using primers r-FAL-1, r-FAL-2, r-VIV-1, and r-VIV-2. The results of the amplified DNA were analysed using electrophoresis and visualized with gel documentation.

Statistical analysis

Data were analysed to describe the frequency distribution and percentage of the tested variables (gender, age, residence, occupation, length of stay in endemic areas, BMI, and malaria infection). Kolmogorov-Smirnov test was used to assess data normality. Chi-square and multiple logistic regression analyses were used to determine the risk factors of anaemia. The independent t-test and Mann-Whitney test were performed to determine the mean difference of Hb between each tested variable mentioned above. Data analysis was performed using SPSS 22.0, and a $p < 0.05$ was considered statistically significant.

Ethical Clearance

This study was approved by The Research Ethics Committee, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia No. 08/10/KEP-FKUAJ/2019.

RESULTS

Prevalence of Malaria and Anaemia

The number of respondents in this study was 510; 148 (29.1%) were infected with malaria, 92 (62.1%) were infected with *P. vivax*, and 56 (37.8%) were infected with *P. falciparum* (Table I). In this study, 278 (54.5%) respondents had anaemia. Anaemia was most prevalent in women (30.6%), compared to men (23.9%). Most respondents were in the younger age group (12-42 years old), accounting for 51.6% of the total respondents. Those who lived in Batu Putih District had the highest number of respondents with anaemia (14.7%). Housewives were found to have the highest prevalence of anaemia (30.6%) and this group also made up the majority (58.6%) of the respondents. Respondents who lived in endemic areas for more than 11 years (42.7%) had the highest prevalence of anaemia. It is interesting to note that 36.1% of the respondents were underweight (BMI <18.5). Anaemia was more common in *P. vivax* infection (11.2%) compared to *P. falciparum* (6.9%) (Table I).

Table I: Sociodemographic characteristics and prevalence of anaemia among respondents (N = 510)

Variable	Hb levels (g/dL)		Total (%)
	Anaemia (%)	Non-anaemia (%)	
Gender			
1. Male	122 (23.9)	89 (17.5)	211 (41.4)
2. Female	156 (30.6)	143 (28.0)	299 (58.6)
Age (years)			
1. 12-42	128 (25.1)	135 (26.5)	263 (51.6)
2. 43-95	150 (29.4)	97 (19.0)	247 (48.4)
Residence (subdistrict)			
1. South Amanatun (API > 5)	41 (8.0)	52 (10.2)	93 (18.2)
2. Central Amanuban (API > 5)	42 (8.2)	43 (8.4)	85 (16.7)
3. South Amanuban (API 1 - ≤ 5)	51 (10.0)	44 (8.6)	95 (18.6)
4. Batu Putih (API 1 - ≤ 5)	75 (14.7)	34 (6.7)	109 (21.4)
5. Oenino (API ≤ 1)	69 (13.5)	59 (11.6)	128 (25.1)
Occupation			
1. Farmer	111 (21.8)	83 (16.3)	194 (38.0)
2. Civil servant	4 (0.8)	0 (0.0)	4 (0.8)
3. Private employee	7 (1.4)	6 (1.2)	13 (2.5)
4. Housewife	156 (30.6)	143 (28.0)	299 (58.6)

CONTINUE

Table I: Sociodemographic characteristics and prevalence of anaemia among respondents (N = 510) (CONT.)

Variable	Hb levels (g/dL)		Total (%)
	Anaemia (%)	Non-anaemia (%)	
Length of stay in endemic areas			
1. <1 years	5 (1.0)	5 (1.0)	10 (2.0)
2. 2-5 years	28 (5.5)	27 (5.3)	55 (10.8)
3. 6-10 years	27 (5.3)	22 (4.3)	49 (9.6)
4. >11 years	218 (42.7)	178 (34.9)	396 (77.6)
BMI			
1. <18,5 (underweight)	114 (22.4)	70 (13.7)	184 (36.1)
2. 18,5 -24,9 (normal)	139 (27.3)	135 (26.5)	274 (53.7)
3. ≥25,0 (overweight & obesity)	25 (3.9)	27 (5.3)	52 (10.2)
Malaria			
1. *P. falciparum* (+)	35 (6.9)	21 (4.1)	56 (11.0)
2. *P. vivax* (+)	57 (11.2)	35 (6.9)	92 (18.0)
3. Negative	186 (36.5)	176 (34.5)	362 (71.0)
Total	278 (54.5)	232 (45.5)	510 (100.0)

Risk Factors of Anaemia in Malarial Infection

Table II showed that age, type of residence, malaria infection, and BMI were associated with low Hb levels (p<0.05). Individuals with positive malaria infections were at greater risk of decreased Hb levels (p<0.05). Respondents in the 12-42 years age group had a lower incidence of anaemia compared to those in 43-95 years age group (p<0.05). Those with a BMI < 18.50 were at risk of anaemia if infected by malaria (p<0.05). Other variables, such as gender, occupation, and length of stay in endemic areas did not show any statistically significant association to anaemia (p>0.05).

Table II: Risk factors of anaemia in malarial infection

Variable	Anaemia (%)	Non-anaemia (%)	χ^2	*df*	*p-value*	*OR*	*CI* (95%) *lower-upper*
Gender							
1. Male	122 (23.9)	89 (17.5)	1.59	1	0.207	1.3	0.9 – 1.8
2. Female	156 (30.6)	143 (28.0)					
Age (years)							
1. 12-42	128 (25.1)	135 (26.5)	7.13	1	0.006*	0.6	0.4 – 0.9
2. 43-95	150 (29.4)	97 (19.0)					
Residence							
1. High endemic	83 (16.3)	95 (18.6)	6.85	1	0.009*	0.6	0.4 – 0.9
2. Low endemic	195 (38.2)	137 (26.9)					
Occupation							
1. Farmers	111 (57.2)	83 (16.3)	0.93	1	0.336	1.2	0.8 – 1.7
2. Non-farmers	167 (32.7)	149 (29.2)					
Length of stay in endemic area							
1. >23 years	136 (26.7)	116 (22.7)	0.06	1	0.808	0.96	0.7 – 1.4
2. ≤23 years	142 (27.8)	116 (22.7)					
Infected by malaria							
1. Positive	92 (18.0)	56 (11.0)	4.92	1	0.026*	1.6	1.1 – 2.3
2. Negative	186 (36.5)	176 (34.5)					
BMI							
1. <18.50	114 (22.4)	70 (13.7)	6.44	1	0.011*	1.6	1.1 – 2.3
2. ≥18.50	164 (32.2)	162 (31.8)					
Total	278 (54.5)	232 (45.5)					

Notes: *χ^2: chi-square, df: degrees of freedom, OR: odds ratio, CI: confidence interval.* Bivariate analysis was conducted using the chi-square test, with a *p-value <0,05* considered statistically significant. 1) Analysis of aged based on median age = 42 years; 2) High endemic residence with API ≥5 ‰; 3) Occupation includes division of farmers and non-farmers; 4) Infected by malaria based on PCR examination results; 5) BMI is measured using weight (kg) / height^2 (m^2)

The results of multivariate analysis with multiple logistic regression tests and modelling showed that four variables (malaria infection, age, residence, and BMI) were associated with a significant reduction in haemoglobin levels (p<0.05)

Malaria Species and Impact on Hb levels

No significant difference in the average Hb levels were observed between the two malarial species. Higher Hb levels were associated with men, high endemic areas and farmers (p<0.05). Based on the Mann-Whitney analysis (Table III), a significant difference in Hb levels was observed between age and BMI groups, with higher Hb levels found in the 12-42 age group and in individuals with a BMI ≥ 18.50 (p<0.05).

Table III: Multivariate analysis (multiple regression logistic) risk factors for anaemia

		Model-3				
No.	**Variable**	***B***	**S.E.**	***p-value***	***95% CI***	**R**
1.	Age	-0.10	0.04	0.03	-0.18 – -0.01	0.21
2.	Residence	-0.12	0.05	0.01	-0.21 – -0.03	
3.	Infected by malaria	0.10	0.05	0.04	0.00 – 0.19	
4.	BMI	0.10	0.05	0.03	0.01 – 0.19	

Notes: *B: unstandardized beta, S.E: standard error, CI: confidence interval, R: correlation coefficient.* Multivariate analysis with multiple logistic regression, conducted three times, showed that variables above contributed 21% to the decrease in haemoglobin levels, with a *p-value <0,05* considered statistically significant.

Table IV: Comparison of mean Hb levels by malaria species and other variables

Variable	**Mean Hb levels (g/dL)**	**SD/SE**	**t**	**df**	***CI***	***p-value***
Malaria species (N=148)						
1. *P. falciparum*	11.13	2.44/0.33	-0.31	146	-0.91 – 0.67	0.761
2. *P. vivax*	11.25	2.29/0.24				
Gender (N=510)						
1. Male	11.90	2.34/0.16	3.47	508	0.32 – 1.16	0.001
2. Female	11.16	2.39/0.14				
Residence (N=510)						
1. High endemic	11.99	2.41/0.18	3.65	508	0.37 – 1.23	0.000
2. Low endemic	11.19	2.34/0.13				
Occupation (N=510)						
1. Farmers	11.92	2.33/0.17	3.38	508	0.31 – 1.15	0.001
2. Non-farmers	11.19	2.39/0.14				

Notes: *SD/SE: standard deviation/standard error, df: degrees of freedom, CI: confidence interval.* The analysis was conducted using an independent t-test, with a *p-value < 0.05* considered statistically significant. The assumption of normality for the data was fulfilled.

Table V: Statistical comparison of median Hb levels by age, length of stay, and BMI

Variable	**N**	**Median**	**Q1**	**Q3**	**Z**	***p-value***
Age						
1. 12 – 42	263	11.80	10.00	13.60	-2.8	0.01
2. 43 - 95	247	11.30	9.50	12.80		
Length of stay in endemic area						
1. >23 years	252	11.70	9.70	13.08	-0.0	0.97
2. ≤23 years	258	11.65	10.00	13.10		
BMI						
1. <18.50	184	11.00	9.13	13.00	-3.0	0.00
2. ≥18.50	326	11.80	10.00	13.20		

Notes: *df: degrees of freedom, CI: confidence interval, Q1: first quartile, Q3: third quartile, Z: a measure of how many standard deviations an element is from the mean of a distribution.* The analysis of significant differences was conducted using the Mann-Whitney U test, with a *p-value < 0.05* considered statistically significant. The assumption of non-normality for the data variables was fulfilled.

DISCUSSION

Malaria remains one of the challenges in eliminating infectious diseases in Indonesia. In this study, malaria infection was 29.1% among 510 respondents. This result was higher than those of other studies in Eastern Indonesia, which reported rates of 18.6% and 17% in Timika, Central Papua Province respectively (16, 17). The high prevalence in this study may be attributed to differences in geographical settings, the number of respondents, and study periods.

The prevalence of *P. falciparum* infection in this study was 37.8% (56/148), while *P. vivax* was 62.1% (92/148); this is in line with a study by Langford et al., who found that the two most common *Plasmodium* species infections in Indonesia were *P. falciparum* (51%) and *P. vivax* (33.3%) (16). A study by Kenangalem et

al. also revealed that *P. falciparum* infection was more prevalent (47.7%) compared to *P. vivax* 37.8% among respondents infected with malaria (17).

In this study, we observed significant association between low Hb levels and malaria infection, age, residence, and BMI. The proportion of anaemia among those infected was 62.2%. A significant relationship between clinical malaria and anaemia was also reported in a study by Mongi et al. at Wori Public Health Centre, North Minahasa Regency (18). Furthermore, Punnath et al. also reported a significant reduction in Hb levels caused by various species of malaria infection (19). Pathogenic mechanisms of anaemia in malaria infection are complex, including rupture of erythrocytes during the release of merozoites, *Plasmodium* destruction of infected erythrocytes, and destruction of uninfected erythrocytes through activation of complement by the reticuloendothelial system in the spleen (20, 21).

The effect of age on Hb levels is influenced by a person's immune system, which develops gradually and matures by 15 years old, whereas after 60 years old, the immune system begins to decline. As a result, when there is exposure to malaria mosquitoes, those above the age of 60 years old are at a higher risk of malaria and anaemia (22). Our findings were consistent with the findings by Stefani et al.'s research at Hanura Health Centre, Pesawaran Regency that revealed older age group who were infected with malaria had lower Hb levels compared to the younger age group.

This current study also revealed that respondents living in low-grade malaria-endemic areas had a higher risk of anaemia than respondents living in high-grade malaria-endemic areas. This was consistent with a recent study from India which also revealed a higher Hb level among those living in high malaria endemic districts, likely due to their dietary habits, particularly the regular consumption of meat products (24).

East Nusa Tenggara has the highest proportion of children under five with poor nutritional status (29.5%) and chronic energy deficiency in women of childbearing age (32.5% in non-pregnant women and 36.8% in pregnant women) (25). The significance between low BMI and high incidence of anaemia is in line with the study by Wirth et al., where the prevalence of anaemia decreases with increasing BMI, especially in the high infection burden group (26). Moreover, a study by Gautam et al. in Nepal showed that women aged 15-49 years with an underweight BMI had a significant prevalence of anaemia (27). Undernutrition increases an individual's susceptibility to viral, bacterial, and parasitic infections. These infections can further worsen the nutritional status, creating a detrimental cycle of undernutrition and infection, including malaria (28).

The difference in mean Hb levels between *P. falciparum* and *P. vivax* infections in this study was not significant. However, average Hb levels were slightly higher in *P. vivax* (11.25 g/dL) compared to *P. falciparum* (11.13 g/dL). Mean Hb levels were also reported to be higher in *P. vivax* than in *P. falciparum* by Douglas et al. These could be due to *P. falciparum* being linked to higher parasitaemia, causing rapid and severe erythrocyte destruction in severe acute malaria. In contrast, *P. vivax* infection usually will cause anaemia in chronic malaria infection. (29, 30). This can be caused by the dormant (hypnozoites) phase in the *P. vivax* cycle, which is not found in *P. falciparum* resulting in chronic malaria.

P. falciparum triggers higher Th-1 activity compared to other malaria species (31). Increased Th-1 activity also promotes the proliferation of B lymphocytes, which produce Immunoglobulin G2 (IgG2), leading to the formation of autoantibodies, such as antiphospholipid, anticardiolipin, and anti-neutrophil cytoplasmic antibodies, which contribute to microvascular complications. The presence of immunoglobulins or complement on the surface of uninfected erythrocytes reduces their lifespan, potentially leading to more severe anaemia (30).

This study had some limitations. Our study did not assess the morphological characteristics of red blood cells, which prevented us from distinguishing between anaemia caused by *Plasmodium* and other types of anaemia. Further research is needed, including longitudinal studies to assess the long-term impact of malaria on Hb levels, investigations into other potential risk factors for anaemia, and intervention studies to evaluate the effectiveness of anaemia prevention strategies in malaria-endemic areas.

CONCLUSION

Malaria infection was associated with lower Hb levels, but no significant difference was found between *P. falciparum* and *P. vivax* infections. Older age and an underweight BMI were significantly linked to reduced Hb levels, while men, farmers, and those in high-endemic areas had higher Hb levels. The findings of this study could be beneficial in predicting the clinical outcomes (degree of anaemia) of malaria infection in different species of malarial parasite, different BMI level, gender and endemicity status. However, a more robust and systematic future study is required to further enhance the clinical value of this study.

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