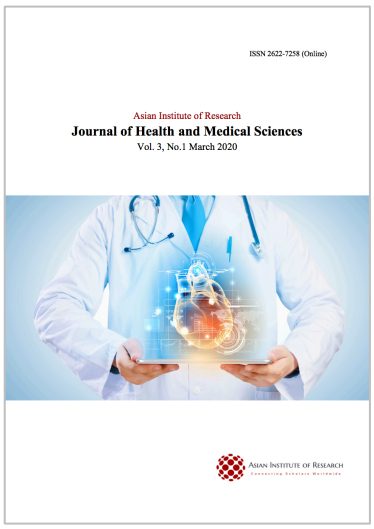

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The Hemoglobin Levels and Characteristics in Malaria Endemic Region in Eastern Indonesia

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Abstract
Introductions: Anemia is the most common symptoms to appear in malaria infection. Hb levels in malaria infection are one of the benchmarks to determine whether or not a patient is anemic as a predictor of the severity of infection. Malarial anemia is capable of causing severe morbidity and mortality especially in children and pregnant women infected. The most prevalent etiology of malaria in Indonesia is *P. falciparum* (52%) and *P. vivax* (38%). There is no specific study about Hb level in the community in an endemic area. Accurate clinical diagnoses are necessary to determine the local specific malaria symptoms. Objectives: This study was conducted to assess the different of Hb levels between malaria *P. falciparum* and *P. vivax* in eastern Indonesia. Methods: This is a cross-sectional study using secondary data in 2013-2014 from 5 sub-districts in South Central Timor Regency. Data was collected and analyzed using bivariate and multivariate analysis with 95% confidence interval (CI) and α= 0.05. Results: Data consisting of 152 respondents were collected. 62,5% (95/152) were infected *P. vivax* and 37.5% (57/152) infected with the *P. falciparum.* Mean of Hb levels from *P. falciparum* 11.130 g/dL and *P. vivax* 11.296 g/dL. T-test resulted *the value of p-value 0.672 (*p-value >0.05) with MD 0.166, 95% CI -0.938-1.606, while multiple linear regression showed regression coefficient -0.059 and p-value 0.876 (p-value >0.05) for Hb levels on *P. falciparum* and *P. vivax*-infected patients. Conclusion: The average level of Hb in *P. falciparum*-infected patients is relatively lower than *P. vivax*-infected patients.

Keywords: East Indonesia, Hemoglobin, Malaria Endemic

1. Introduction

Currently, there are four of five species of *Plasmodium* which can infect human in Indonesia; *P. falciparum, P. vivax, P. malariae,* and *P. knowlesi,* while the *P. ovale* until uptodate never been reported occur in Indonesia (Direktorat Jenderal Pencegahan dan Pengendalian Penyakit, 2018; Suwandi, Asmara, & Kusnanto, 2014).

According to World Malaria Report in 2018, there are 219 million cases of malaria all over the world. The approximate estimation of death caused by malaria is 435,000 deaths, with an estimation of death in the Southeast Asia region is 19,700 deaths (World Health Organization, 2018). The most prevalent etiology of malaria in Indonesia is *P. falciparum* (52%) and *P. vivax* (38%) (Direktorat Jenderal Pencegahan dan Pengendalian Penyakit, 2018). Indonesian Annual Parasite Incidence (API) by the National Survey in 2018 was 0.4‰, with three provinces most contributed to malaria are: Papua, West Papua, and East Nusa Tenggara with the API >5‰ population (*Riset Kesehatan Dasar 2018*, 2018).

The clinical manifestations of malaria will usually show up 8-25 days post-infected in the form of recurrent fever, headache, fatigue, nausea, vomit, shiver, and anemia (Chakraborty, 2016). One of the most common complications of malaria is severe anemia with Hemoglobin (Hb) levels <5 g/dL in high endemic and Hb levels <7 g/dL in mild-moderate endemic (*Buku Saku Tatalaksana Malaria*, 2017; Natalia, 2015). Anemia is defined by the condition of Hb levels on blood that is less than the normal level (Cappellini & Motta, 2015). According to World Health Organization (WHO), the normal Hb level of men is ≥13 g/dL, non-pregnant women ≥12 g/dL, and pregnant women ≥11 g/dL (World Health Organization, 2011). The signs and symptoms of anemia are one of the bases of diagnosing malaria. One of the diagnostic criteria of severe malaria based on WHO is the presence of severe anemia with Hb levels <7 g/dL (*Buku Saku Tatalaksana Malaria*, 2017). The severity depends upon patient-specific characteristics as well as parasite-specific characteristics (Saxena, 2017).

Anemia in malaria may occur by several causes such as the destruction of *Plasmodium* infected-erythrocytes by reticuloendothelial, imbalance of cytokines caused by the hyperstimulation of the immune system, and/ or erythropoiesis suppression in bone marrow (Ahmad et al., 2014; Paniker, 2013). Every *Plasmodium* species has a different characteristic of infected erythrocytes. *P. falciparum* can infect old and young erythrocytes, different to *P. malariae* which can only infect old erythrocytes, and also *P. vivax* and *P. ovale which* can only infect young erythrocytes (A. Srivastava et al., 2015).

Study in M. Djamil Central General Hospital, Padang, West Sumatera which obtained p-value 0.02, concluded that there is a significant difference in the Hb levels of the patient infected by *P. falciparum* and *P. vivax* (Afdhal, Nurhayati, & Julizar, 2014). However, the study conducted in Lindimara Christian Hospital, East Sumba, East Nusa Tenggara which obtained p-value 0.523, concluded that there is not any significant difference in Hb levels of the patient infected by *P. falciparum* and *P. vivax* (Irawan, Merry, & Wuryaningsih, 2017).

Accurate clinical diagnoses are necessary to determine the local specific malaria symptoms. Health officers then may design a health education for the community that stresses how clinical malaria typically occurs in that community (Elyazar, Hay, & Baird, 2011). The lack of research talking about Hb levels on patient infected by each *Plasmodium* species and the different result from earlier research, makes the research on differences Hb levels caused by *P. falciparum* and *P. vivax* in a wider population and endemic areas is need to be done, one of which is in South Central Timor Regency. This research will assess the Hb levels and the characteristic among community in malaria endemic region in eastern Indonesia.

2. Method

2.1 Location and period of time

This is a cross-sectional study part of study done by Hutagalung *et al.* (2018) (Hutagalung, Kusnanto, Sadewa, & Satyagraha, 2018). The research was conducted on August 2013 until September 2014 from 5 sub-district of South Central Timor Regency, East Nusa Tenggara Province based on API: Amanatun Selatan (API >5‰), Amanuban Tengah (API >5‰), Amanuban Selatan (API 1-5‰), Batu Putih (API 1-5‰), dan Oenino (API <1‰).

2.2 Data Collection

A total of 555 respondents were selected from the study master data, only completed data were selected in to this study, such; demography, gender, age, and the malaria-confirmed data by only single infection between *P. falciparum* and *P. vivax* will be analyzed in this study, while for the other incomplete data will be rejected. The

previous research subject was obtained by systematic random sampling based on the household data from each sub-district.

The Hb levels were tested by using the HemoCue Hb test, Helsinborg, Sweden (HemoCue, 2013). The malaria etiologic agents-species is identified by using the microscopic thick and thin blood smears (Giemsa 3-5%) using protocols from the Ministry of Health and the WHO (World Health Organization, 2009). Identification the four species of *Plasmodium (P. falciparum, P. vivax, P. ovale,* and *P. malariae)* were detected that they used the nested Polymerase Chain Reaction (nPCR) method (Promega Corporation, 2014). The nPCR condition followed the protocol of Snounou *et al* (1993) (Snounou, Viriyakosol, Jarra, Thaithong, & Brown, 1993).

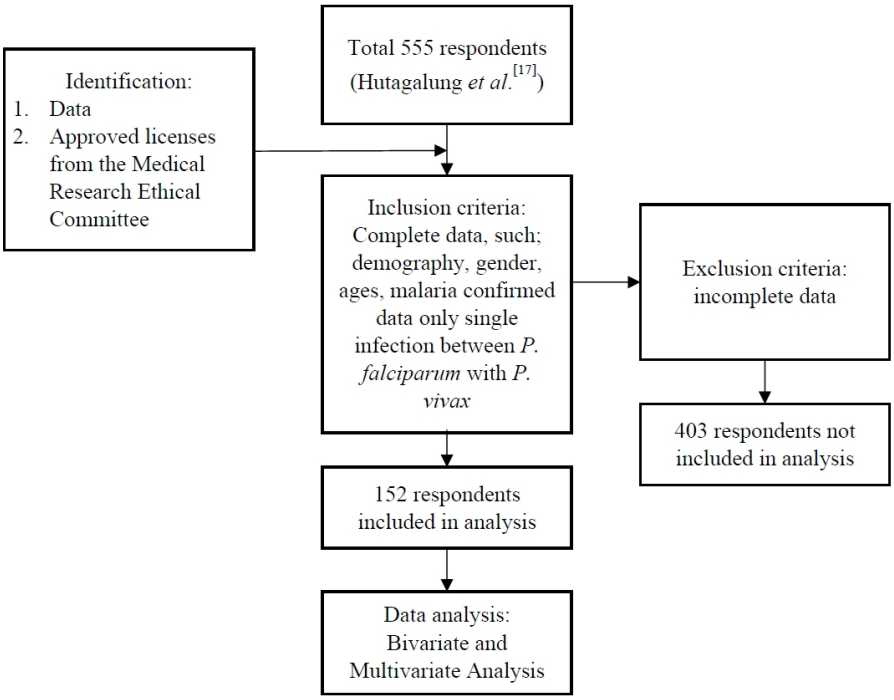

Figure 1. Research Flowchart

2.3 Data analysis

The data will be analyzed using the IBM® SPSS® 25th version software. Characteristics analysis only compared between *Hb levels* higher and lower average group. Data analysis using t-test and multiple linear regression to analyze the differences in Hb levels between *P. falciparum* vs *P. vivax*-infected patients, sex group, and endemic areas status. P-value >0.05 indicates no difference in Hb levels, and a p-value <0.05 indicates there is a difference in Hb levels.

This study has been approved by the Medical Research Ethical Committee, Faculty of Medicine, Universitas Padjadjaran, with ethic license number 663/UN6.KEP/EC/2019.

3. Results

3.1 Characteristic of respondents

Total of 152 respondents were collected for this research based on the inclusion and exclusion criteria. The average value of Hb levels among respondents in this study is 11.23 g/dL, therefor classified data to ≤11.23 and >11.23 group.

Table 1 shows the characteristics of the respondents. The *number of males are less than females in Hb levels* ≤11.23 group*, but the* number of males are more than females in *Hb levels >11.23 group*. The most prevalent age in *Hb Levels* ≤11.23 group is the 40-49-year-old group, while, in *Hb levels >11.23 group* is the 30-39-year-old group. From 5 sub-district that have been chosen as the research location, the largest *Hb Levels* ≤11.23 group are located at Oenino, and the *Hb levels >11.23 group* are located in Batu Putih. *Hb Levels* ≤11.23 group were

found more in sub-district with API index >5 ‰, but *Hb levels >11.23 group* were found more in sub-district with API index 1-5‰. Timor tribe was the largest tribe that *Hb Levels* ≤11.23 *and Hb levels >11.23 group.* The number of *P. falciparum* more than *P. vivax* in *Hb levels ≤11.23 group, on the contrary, the number of P. vivax is more than P. falciparum in Hb Levels* >11.23 group.

Table 1. The Distribution of the Respondents (n=152)

Characteristics	Hemoglobin levels		Total n=152 (%)
	Hb ≤11.23 g/dL n=75 (%)	Hb >11.23 g/dL n=77 (%)	
Gender			
Male	24 (32.0)	43 (55.8)	67 (44.1)
Female	51 (68.0)	34 (44.2)	85 (55.9)
Age (year old)			
<20	3 (4.0)	4 (5.2)	7 (4.6)
20-29	7 (9.3)	2 (2.6)	9 (5.9)
30-39	12 (16.0)	26 (33.8)	38 (25.0)
40-49	22 (29.3)	17 (22.1)	39 (25.7)
50-59	18 (24.0)	18 (23.4)	36 (23.7)
≥60	13 (17.3)	10 (13.0)	23 (15.1)
Sub-district			
Amanatun Selatan	11 (14.7)	11 (14.3)	22 (14.5)
Amanuban Tengah	17 (22.7)	18 (23.4)	35 (23.0)
Amanuban Selatan	10 (13.3)	11 (14.3)	21 (13.8)
Batu Putih	15 (20.0)	22 (28.6)	37 (24.3)
Oenino	22 (29.3)	15 (19.5)	37 (24.3)
Level of API (‰)			
API >5	28 (37.3)	29 (37.7)	57 (37.5)
API 1-5	25 (33.3)	33 (42.9)	58 (38.2)
API <1	22 (29.3)	15 (19.5)	37 (24.3)
Ethnicity			
Timor	68 (88.3)	71 (94.7)	139 (91.4)
Sabu	2 (2.6)	1 (1.3)	3 (2.0)
Rote	7 (9.1)	3 (4.0)	10 (6/6)
Plasmodium sp.			
P. falciparum	32 (42.7)	25 (32.5)	57 (37.5)
P. vivax	43 (57.3)	52 (67.5)	95 (62.5)

Note. API= Annual Parasite Incidence, Hb= Hemoglobin, Sp.= Species

Table 2 shows the bivariate analysis of Hb levels with characteristics of respondents. The results obtained the results of gender has a p-value of 0.003 (p <0.05) which shows there are significant results, while other variable did not show significant results.

Table 2. Bivariate Analysis

Characteristics	Hemoglobin levels		Total n=152 (%)	P-value (OR)	95% CI	
	Hb ≤11.23 g/dL n=75 (%)	Hb >11.23 g/dL n=77 (%)			Lower	Upper
Gender						
Male	24 (32.0)	43 (55.8)	85 (55.9)	0.003	0.192	0.721
Female	51 (68.0)	34 (44.2)	67 (44.1)	(0.372)		
Age						
≤45-year old	38 (50.7)	43 (55.8)	81 (53.3)	0.522	0.651	2.331
>45-year old	37 (49.3)	34 (44.2)	71 (46.7)	(1.231)		
Subdistrict						
Risk areas	28 (37.3)	29 (37.7)	57 (37.5)	0.967	0.526	1.956
No risk areas	47 (62.7)	48 (62.3)	95 (62.5)	(1.014)		
Level of API				0.967	0.526	1.956
High endemic	28 (37.3)	29 (37.7)	57 (37.5)	(1.014)		

Low endemic	47 (62.7)	48 (62.3)	95 (62.5)			
Ethnicity						
Timorese	71 (94.7)	68 (88.3)	139 (91.4)	0.161	0.125	1.447
Non-Timorese	4 (5.3)	9 (11.7)	13 (8.6)	(0.426)		
Plasmodium sp.						
P. falciparum	32 (42.7)	25 (32.5)	95 (62.5)	0.194	0.799	2.998
P. vivax	43 (57.3)	52 (67.5)	57 (37.5)	(1.548)		

Note. 95% CI= 95% Confidence Interval, OR= Odd Ratio

3.2 Analysis of Hb levels

Table 3 shows the t-test analysis for Hb levels. The Mean and standard deviation of Hb levels from *P. falciparum* and *P. vivax*-infected patients. The mean Hb levels from *P. falciparum*-infected patients (11.130 g/dL) tend to be lower than *P. vivax*-infected patients (11.296 g/dL). The mean difference value was -0.166, it means that the Hb level of patients infected with *P. falciparum* is lower by 0.166 g/dL than the Hb level of patients infected with *P. vivax*. *Based on t-test, the value of p-value 0.672,* indicates there is no difference in Hb levels between *P. falciparum* and *P. vivax*-infected patients.

The mean and standard deviation of Hb levels from *male and female patients.* The mean Hb levels from *male* patients (11.931 g/dL) tend to be higher than female patients (10.684 g/dL). The mean difference value was 1.247, it means that the Hb level of male patients is higher by 1.247 g/dL than the Hb level of female patients. *Based on t-test, the value of p-value 0.001,* indicates there is difference in Hb levels between male and female patients.

The mean and standard deviation of Hb levels from between patients live in low endemic area and high endemic area. The mean Hb levels from patients live in high endemic area (11.421 g/dL) tend to be higher than patients live in low endemic area (10.121 g/dL). The mean difference value was 0.300, it means that the Hb level of patients live in high endemic areais higher by 0.300 g/dL than the Hb level of patients live in low endemic area. *Based on t-test, the value of p-value 0.443*, indicates there is no difference in Hb levels between patients live in low endemic area and high endemic area.

Table 3. T-test Analysis for Hemoglobin Levels

Characteristics	n	Mean	SD	MD	95% CI		P-value
					Lower	Upper	
Plasmodium sp.							
P. falciparum	57	11.130	2.418	-0.166	-0.938	0.606	0.672
P. vivax	95	11.296	2.276				
Gender							
Male	67	11.931	2.146	1.247	0.522	1.973	0.001
Female	85	10.684	2.325				
Level of API							
High endemic	57	11.421	2.612	0.300	-0.470	1.071	0.443
Low endemic	95	11.121	2.141				

Note. 95% CI= 95% Confidence Interval, API= Annual Parasite Incidence, MD= Mean Difference, SD= Standard Deviation, Sp.= Species

Table 4 shows the results of the final steps analysis using multiple linear regression test with the entered method. The regression coefficient value was -0.059, it means after adjusted for gender and Level of API that the Hb levels of patients infected with *P. falciparum* is lower by 0.059 g/dL than the Hb level of patients infected with *P. vivax*. P-value was 0.876, it means that there was no difference in Hb levels between *P. falciparum* and *P. vivax*-infected patients.

Table 4. Multivariate Analysis

Variable	B	t	P-value	95% CI	
				Lower	Upper
Constant	11.410	17.408	0.001	10.115	12.705
Types of *Plasmodium*	-0.059	-0.156	0.876	-0.808	0.690

Gender	1.294	3.485	0.001	0.560	2.027
Level of API	-0.446	-1.171	0.244	-1.198	0.307

Note. 95% CI= 95% Confidence Interval, B= Coefficients regression

4. Discussion

The result of this research indicates that there are no differences in Hb levels in *P. falciparum* and *P. vivax*-infected patients. These findings are consistent with the research conducted in Tak Province, Thailand by Kotepui *et al.* (2015) which obtained a p-value of 0.21 (Kotepui et al., 2015), research conducted in India and Arab Saudi by Khan *et al.* (2014) which obtained a p-value 0.233 (Khan & Zakai, 2014), and research conducted in East Sumba, East Nusa Tenggara by Irawan *et al.* (2017) which obtained a p-value 0.523 (Irawan et al., 2017). It showed that there were no differences in Hb levels in *P. falciparum* and *P. vivax*-infected patients (Irawan et al., 2017; Khan & Zakai, 2014; Kotepui et al., 2015). In contrast to the research conducted in Vellore, Tamil Nadu, India by Mitra *et al.* (2015) which obtained a p-value 0.03 (Mitra, Abhilash, Arora, & Miraclin, 2015), and research conducted in Padang, West Sumatera by Afdhal *et al*. (2014) which obtained a p-value 0.02 (Afdhal et al., 2014). Their research showed that there were differences in Hb levels between *P. falciparum*-infected patients and *P. vivax*-infected patients (Afdhal et al., 2014; Mitra et al., 2015). There were differences in Hb levels due to differences in the pathogenesis of the disease. In *P. falciparum* produces more Th-1 than other *Plasmodium*, so the disease is more severe (Afdhal et al., 2014).

East Nusa Tenggara Province is an endemic malaria area that also stands in third place of area with the highest malaria prevalence in Indonesia (*Riset Kesehatan Dasar 2018*, 2018). In the endemic area, protection towards malaria can be obtained by repeated exposure. Individual who lives in the endemic area will be able to tolerate high parasite level without manifest any sign and symptoms. This shows that in the endemic area, the threshold to reach the border of clinical malaria is higher than in non-endemic areas (Ademolue, Aniweh, Kusi, & Awandare, 2017).

In this research, the results showed that there were no significant differences in Hb levels. That is because the respondents in this study were healthy populations and did not suffer from chronic diseases. However, in theory, the decrease in Hb levels in malaria infection can be caused by several things, such as: malaria species that infect, number of parasites, duration of infection, and individual immune status (Khan & Zakai, 2014). From the results of this research, researchers can conclude that in certain conditions, individuals who suffer from malaria and live in malaria endemic areas have no differences in Hb levels.

From the average Hb levels, there is a decrease in Hb levels in *P. falciparum* and *P. vivax*-infected patients. Hb levels in *P. falciparum*-infected patients is relatively lower than in *P. vivax*-infected patients. This shows that it is true that in malaria, infection from *Plasmodium* will attack red blood cells which later will make the red blood cell number is low and become anemia (Cox, 2010).

Even though there are many unexplainable complex factors that involve in the progress of anemia during malaria infection, there are several mechanisms that may responsible for malaria, they are; (1) *Plasmodium* which already invades red blood cell will digest the Hb with parasite protease into small fragments and produce hemozoin that later will projects antigenic molecule in red blood cells membrane and in the end the red blood cell will be phagocytized by circulating macrophage. (2) During the *Plasmodium* infection stage, schizont will destroy infected-red blood cell to release merozoites and hemozoin to blood circulation where hemozoin will sediment in uninfected-red blood cell and provoke lipid peroxidation in red blood cell membrane then will lead to cell deformity and hemolysis. (3) Hemozoin deposition in bone marrow shows inhibition of erythropoiesis and change in dyserythropeietic. (4) Hemozoin will increase TNF- α secretion, where pro-inflammatory cytokines like TNF-α have been proven to show the inhibition in all stages of erythropoiesis (Ihekwereme, Esimone, & Nwanegbo, 2014).

The result of this research indicates that there are significant differences in Hb levels in males and females. This due to differences in the direct effects of sex hormones, such as estrogen and androgens. Women have mean levels of approximately 12% lower than men (Murphy, 2014). Hb levels <11.23 g / dL are found in the age

group of 40-49 years, this is because the majority of respondents are the age group above 30 years. Other than that, age can affect the Hb level, where increasing age can decrease clinical protection and cause a greater decrease in Hb levels (Siqueira et al., 2014). Based on population data in 2016, the population of South Central Timor District was 461,681 people, with the majority being the Timorese tribe. This is the reason why this research is dominated by the Timorese tribe (*Provinsi Nusa Tenggara Timur Dalam Angka 2017*, 2017).

The limitation of this research is the data collection that was only done once. To see the difference in Hb levels more significantly, it is necessary to take continuous data collection (cohort study) since with continuous data collection, the pathogenesis of the disease will be clearer to see and also the difference in Hb levels. In this research, Hb tests were conducted by using HemoCue which has variated sensitivity and specificity towards the type of venous or capillary blood sample. This research used capillary blood which based on research has a sensitivity of 56-94.7% and specificity of 80.1-100% (T. Srivastava, Negandhi, Neogi, Sharma, & Saxena, 2014). The use of HemoCue with capillary blood also based on research results in an average value of Hb levels in the blood higher than veins (T. Srivastava et al., 2014; Yadav, Jacob, Ahamed, Mandal, & Kant, 2018). To obtain a better result, the next research may use a tool that has better sensitivity and specificity than HemoCue, for example, an automatic hematology analyzer (T. Srivastava et al., 2014). An automatic hematology analyzer can examine many additional parameters which later may result in more information given (Chhabra, 2018).

This research concludes that the average level of Hb in *P. falciparum*-infected patients is relatively lower than *P. vivax*-infected patients.

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