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The parasite density and erythrocyte sedimentation rate on patients with uncomplicated tropical Malaria In two community health centre of West Lombok

Kadeq Novita Prajawanti[1a*], Siti Zaetun[2], Pancawati Ariami[2]
[1]Graduate School of Immunology, Postgraduate School, Universitas Airlangga, Surabaya.
[2]Department of Medical Laboratory Technology, Poltekkes Kemenkes Mataram
[a]kadeqnprajawanti@gmail.com
* corresponding author

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ABSTRACT

Malaria infections are often associated with the activation of coagulation and fibrinolytic systems. An increase number of fibrinogen levels in severe malaria and the increase of fibrinogen also stimulated the increase of erythrocytes sedimentation rate. The aim of this study is to find out about the effects of high parasitemia to erythrocyte sedimentation rate in patients with uncomplicated tropical malaria. Samples are collected using the *Purposive Samplings* method. To determined the effect of parasite density to erythrocytes sedimentation rate (ESR) levels (mm/h), the data were analyzed using the *Non-Parametric Kruskal Wallis* test (α=0,05). From 8 samples, 2 subjects (25%) has ++ (2+) densities with 35 and 46 mm/h; 3 subjects (37,5%) has +++ (3+) densities with 10, 65 and 70 mm/h; and also 3 other subjects (37,5%) has ++++ (4+) densities with 21, 44, and 70 mm/h. The Non-parametric Kruskal Wallis test shows that p-value 0,932 >α, means there is no effect of parasitic density on ESR levels in patients with uncomplicated tropical malaria. Using the ESR as the only-main biomarker in assessing the severity of malaria is an inaccurate idea because the ESR is more likely a non-specific test, therefore another blood test is needed to establish a diagnosis of malaria severity.

INTRODUCTION

Malaria is a health problem that can cause death. This disease is also endemic in most parts of Indonesia.[1] Malaria is an infectious disease also occour as acute or chronic infection, caused by Plasmodium parasites infects the erythrocytes cells.[2] Tropical malaria is malaria caused by *Plasmodium falciparum*.[3] Malaria sufferers without complications usually show symptoms of main high fever which is paroxysmal accompanied by chills, sweating, and headache. In addition, fatigue, anorexia, back pain, myalgia, pale, and vomiting are often found.[4]

Malaria is a parasitic disease that is quite high in the world and causes death in 40% of the population (around 2.4 billion people) in more than 100 countries. Malaria is caused by Plasmodium spp., particularly it affects in tropical and subtropics populations, and especially in developing and under-developed countries.[5]

In 2010 there were 229,819 cases of malaria in Indonesia.[6] Nationally, malaria morbidity rates during 2005-2015 tended to decrease, from 4.1 per 1,000 at-risk population in 2005 to 0.85 per 1,000 at-risk population in 2015.[7] Data from the West

Lombok Health Service in 2015 showed that Malaria is still one of the health problems in West Lombok Regency. Clinical malaria cases in 2015 showed 201 malaria positive cases, an increase from 198 cases the previous year. The highest number of positive malaria cases were still found in the coastal and mountainous areas, namely the Sekotong Community Health Center with 86 positive malaria cases, followed by 44 cases in the Meninting area and 23 in the Gunung Sari area.[8]

To be able to see the presence of *Plasmodium* parasites in the patient's blood, malaria blood preparations need to be made. Furthermore, it was stained with Giemsa staining.[9] Thick blood preparations are usually used to count parasites quantitatively so that the severity of malaria can be identified in a person.[10]

Blood coagulation activation is frequently found in patients with malaria,[11] thrombocytopaenia and haemostatic alterations lead to increased activation of coagulation cascade and fibrinolytic system.[12] Previous study by Mohanty (in Adeayo & Adedire, 2012) showed that in severe malaria there have been consistent elevated concentration of fibrin degradation product, usually with increased level of fibrinogen and suggesting increased fibrinogen turnover.[13] Fibrinogen is an acute phase protein, during acute inflammation, plasma fibrinogen can increased over 7 mg/mL.[14] Both fibrinogen and fibrin are essential for hemostasis, thrombosis, and several other biological functions and also pathological conditions.[15] An increase in the concentration of plasma protein contents such as fibrinogen tends to increase the value of the Erythrocytes sedimentation rate (ESR).[16]

ESR is a measurements of erythrocyte sedimentation velocity, describing plasma composition as well as the ratio of erythrocytes to plasma.[17] Although ESR is not a specific marker for inflammation, nowadays, it frequently used by clinicians to help make a diagnosis and to help in evaluating or monitoring patients with chronic diseases. ESR is still widely used as an examination of acute phase response screening and monitoring test for infection, autoimmune and malignant diseases.[18]

An increase in fibrinogen levels in severe malaria,[13] and an increase in fibrinogen tends to increase the rate of ESR.[16] Researcher were interested in conducting research on the effect of parasitic density on ESR levels in patients with uncomplicated tropical malaria, where the parasitic density greatly determines the level of parasitic infection in determining the severity of malaria infection.

METHODS

Research design

This research is analytic observational by using cross sectional design. The way to take samples is done purposively based on certain considerations made by the researchers themselves.[19] So that the sample to be used comes from respondents who were positive suffering from Tropical malaria without complications and exactly as the criteria that had been made by researcher. The following criteria are: a). willing to be the subject of research; b). positively suffer from tropical malaria; c). didn't consuming anti-inflammatory drugs; d). didn't suffering from Malaria with complications, such as: tuberculosis, hepatitis, DHF, myocardial infarction and other heart diseases, malignancy, kidney failure, other acute and chronic infections.

Population and samples

Population of this study were all patients who showed clinical symptoms of malaria in two *Community Health Centre* (Puskesmas [Indonesian: Pusat Kesehatan Masyarakat]) of West Lombok i.e. Puskemas Meninting and Puskesmas Gunung Sari in March to June 2017. The samples to be used were all patients who tested positive for tropical malaria without complications who examined themselves at Puskesmas Meninting and Puskesmas Gunung Sari from March to June 2017.

**Corresponding author: kadeqnprajawanti@gmail.com*

Malaria Parasites Counting

In the examination of thick blood when parasites are found in various stages of development, parasitic density is counting by the number of *Plasmodium* parasites in the form of all stages in 100 high-power fields (HPF). Then the number of parasites is reported as follows[6]:

(-) = Negative (there is no parasite/100 HPF)
(+) = Positive 1 (1-10 parasies/100 HPF)
(++) = Positive 2 (11-100 parasies/100 HPF)
(+++) = Positive 3 (1-10 parasites/ 1 HPF)
(++++) = Positive 4 (>10 parasites/1 HPF)

Measurement of ESR Levels using Westergren Method

Prepare the tools such as: Westergren tube and rack, blood samples, bulb and Sodium Citrate 3.8%. Blood samples were collected using EDTA.K3 Onemed vacular tube.The westergren method is carried out by mixing 2 ml of venous blood and 0.5 ml of sodium citrate. Blood and sodium citrate mixtures then put into a Westergren-Katz tube up to 200 mm. The tube is left vertically on the shelf for 1 hour at room temperature. The rate of erythrocyte deposition from a blood sample is expressed in millimeters per hour (mm / hour).

The following are considered normal ESR test results: Adults (under 50 y.o) men: <15mm/h, women: <20mm/h; adults (more than 50 y.o) men: ≥ 20mm/h, women: ≥30mm/h.[6]

Data Analysis

Data obtained from the results of parasitic density and ESR levels with Westergren method is processed and analized using SPSS 16 Software then to determine whether there is an effect of parasitic density to the ESR levels, non-parametric *Kruskal Wallis* test will be carried out, with α 0.05.

RESULT AND DISCUSSION

Demographic Characteristics of Research Subjects

In general, the results of research conducted in March to June 2017 based on location and the number of samples obtained and the density of parasites are found in the results presented in Table 1.

Table 1 Distribution of sampling locations and parasite counts and densities

No	Locations	Frequency		Parasites Densities	Parasites count
		Amounts	(%)		
1	Puskesmas Meninting	2	25	++	112/100 HPF
				++	102/100 HPF
2	Puskesmas Gunung Sari	6	75	+++	239/100 HPF
				++++	950/50 HPF
				+++	421/100 HPF
				++++	891/50 HPF
				++++	751/50 HPF
				+++	374/100 HPF

From table 1 it can be seen that there are 2 subjects (25%) sufferers of tropical malaria came from Puskesmas Meninting with the same density, both has 2+; as many as 6 subjects (75%) of malaria patients came from the Puskesmas Gunungsari with 3 subjects has 3+ and 3 others has 4+.

**Corresponding author: kadeqnprajawanti@gmail.com*

Based on data from the West Lombok health office, the most malaria cases in west Lombok located in the Gunung Sari and Meninting regions[8], but in this study only 8 samples were found suitable to the research criteria. In addition, researchers assume that due to March-June was not the rainy season, malaria cases get decrease. Hence malaria are expected to influence by temperature, rainfall, humidity due to climatic change.[20]

Parasite Density and ESR Levels

From the research conducted in March to June 2017, the results of parasitic density and ESR leves shown in table 2.

Tabel 2. Results of parasites densities and ESR levels

Parasites Densities	ESR levels (mm/h)
Mild (++)	35
	46
Moderate (+++)	10
	65
	70
High (++++)	21
	44
	70

From table 2 it is known that 2 subjects (25%) had parasitic density ++ (2+); 3 subjects (37.5%) had parasitic density +++ (3+); and 3 other subjects (37.5%) had a parasitic density ++++ (4+). The lowest sediment rate is 10 mm/hour while the highest blood sedimentation rate is 70 mm/hour.

Researchers assume that although a large number of parasites are found in thick drops examination, a person does not always have a high ESR levels because the production of fibrinogen is also strongly influenced by the patient's immune system and is not only determined based on the level of parasitemia. Who convey that some immunity to malaria infection can reduce the acute clinical symptoms that are experienced.[21] Conversely, an increase in anti-parasitic immunity accompanied by a level of immunity will appear to be free of infection in areas with moderate to high transmission. Today, parasitic densities are often found to be low and sometimes not detected microscopically and most adults who are infected do not show clinical symptoms but this parasitemia condition without symptoms can affect all age groups.[21]

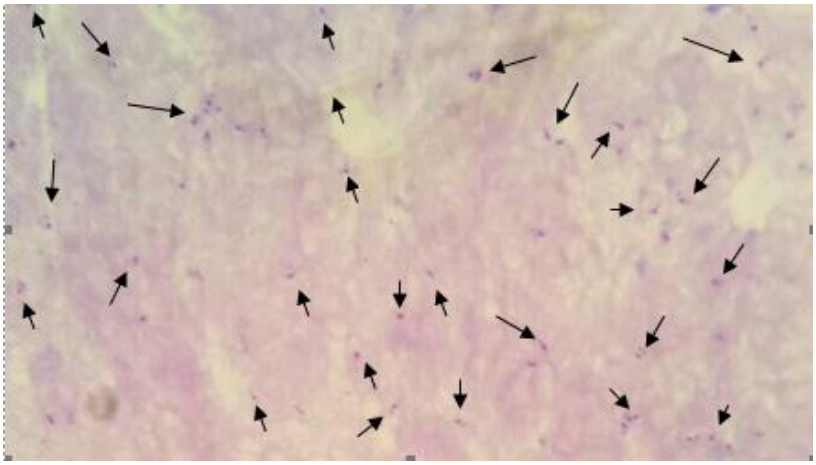

Figure 1. Trophozoites stage of *Plamodium falciparum*

**Corresponding author: kadeqnprajawanti@gmail.com*

Representative picture of *Plasmodium falciparum* trophozoit stage (Figure 1) observed on Giemsa-stained thick blood smears. In *P. falciparum* infections, trophozites characterized by non-enlarged erythrocytes, double chromatin points and multiple infections per erythrocyte, can be seen as ring-like form, early tropozoites and crescent-like form.[22]

Statitiscal Analysis

To determine whether there is an effect of parasitic density on the ESR levels the Non-parametric Kruskal Wallis test has been used.

Tabel 3. Non-parametric Kruskal Wallis test

	ESR (mm/h)
Chi-Square	.141
df	2
Asymp. Sig.	.932

Tabel 3 shows the statistics test of ESR and parasite densities. P value of the test is 0.932 > α means there is no effect of parasitic density on ESR levels in patients with uncomplicated tropical malaria. Based on the results of the study using 8 subjects with uncomplicated malaria tropica, it was found that there was no effect of parasitic density on ESR levels in patients with uncomplicated tropical malaria. High parasitic counts or parasitemics do not show a significant increasing in ESR. High ESR level can be caused by inflammation that occurs during an infection or can also be caused by the presence of inclusion bodies.[23] Even thought ESR westergren method is an unspecified examination but nevertheless this westergren is recognized as a standard method for checking blood sedimentation rates and is still very widely used at this time.[24] ESR is a non-specificity test, because it is tend to be falsely positive (elevated with or without inflammation) compared to C-reactive protein test. In addition, a slow ESR response to acute phase reactions can cause false negatives in the early inflammatory process.[25]

Although there is no relation between parasitic density and severity of malaria, some individuals with high parasitic densities do not have severe symptoms of pain while other individuals who have low parasitic density actually appear to have fatal symptoms of illness. When a person suffers from malaria even with a low parasitic density the person is most likely to have severe malaria and that is enough to determine that he has hyperparasitemia.[26] Protective immunity against malaria is slow to form. This immunity is only obtained after individuals grow up and after being infected with parasites repeatedly. Therefore, immunity to malaria is only possessed by people who live in stable endemic areas that are exposed to parasites almost daily.[27]

In addition to the activity of proteins as an acute phase that play a role in the inflammatory process, as well as the immunity of patients with different malaria, ESR levels are also strongly influenced by technical factors. In this case the researchers argue that there are some things that are technical in nature that affect the value of ESR. This error could have occurred during the process of taking blood where the process was carried out by the relevant health center staff. Blood sampling is done with a 3cc syringe and then blood is filled from the syringe into the EDTA vaccutainer tube by piercing through the rubber tube. This can cause hemolysis of red blood cells if pressure is applied to the syringe which results in blood samples becoming more fluid and changing the shape of erythrocytes. In addition, if the blood volume is not proportional to the volume of anticoagulants, it will increase the risk of errors in ESR examination.

There are some things that need to be considered in the ESR examination that can affect the accuracy of the results of the examination so that it can be a factor in the

**Corresponding author: kadeqnprajawanti@gmail.com*

inspection process, namely excessive anti-coagulant concentration that can increase the ESR levels.[28] The comparison of blood with anticoagulants must be precise if the use of EDTA more than 1 mg/ml of blood will affect the shape of erythrocytes so that the erythrocytes will shrink which will cause ESRs to be low.[29] Then, the researcher assumes that a relatively small number of samples can also make no significant effect between the parasitic density on the ESR levels because, according the larger the sample size, the less random errors that exist in the study.[30] Therefore, the limitation in this study is the lack of research samples being used so as to allow for some errors. Further research is expected to be able to use a larger number of samples so that the results of the study are more trustworthy.

CONCLUSION

There was no effect between *Plasmodium falciparum* parasite density with ESR levels. However, further research with a larger number of samples is needed to improve research accuracy. Suggestions that researcher can convey is that research on malaria should be carried out in the rainy season, around January-March, because at that time the incidence of malaria tends to increase. Using the ESR as the only-main biomarker in assessing the severity of malaria is an inaccurate idea because the ESR is more likely non-specific test, therefore another blood test is needed to establish a diagnosis of malaria severity.

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**Corresponding author: kadeqnprajawanti@gmail.com*

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**Corresponding author: kadeqnprajawanti@gmail.com*