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RESEARCH

DIAGNOSTIC TEST OF HEMATOLOGY PARAMETER IN PATIENTS SUSPECT OF MALARIA

(Uji Diagnostik Tolok Ukur Hematologi di Pasien Terduga Malaria)

Ira Ferawati¹, Hanifah Maani², Zelly Dia Rofinda³, Desywar⁴

ABSTRAK

Malaria masih merupakan masalah kesehatan di daerah tropis dan sub tropis terutama Asia dan Afrika dengan angka kesakitan dan kematian yang tinggi. Parasit masuk ke dalam darah selain menimbulkan gejala klinis berupa demam, juga diduga memicu terjadinya perubahan hematologi antara lain monositosis dan trombositopenia. Penelitian ini bertujuan mengetahui uji diagnostik tolok ukur hematologi di pasien terduga malaria. Penelitian uji diagnostik potong lintang ini dilakukan terhadap 60 orang pasien terduga malaria yang memenuhi patokan kesertan dan nonkesertan masa waktu Juli 2015 sampai Maret 2016 di Instalasi Laboratorium Sentral RSUP. Dr. M. Djamil Padang, Rumah Sakit Tingkat III Reksodiwiryo Padang, Puskesmas Barung Belantai Kabupaten Pesisir Selatan, Rumah Sakit Hanafie Kabupaten Bungo, Rumah Sakit Sultan Thaha Saifuddin dan Puskesmas Rimbo Bujang Kabupaten Tebo. Tolok ukur yang diperiksa selain mikroskopis malaria adalah hitung monosit dan trombosit. Analisis statistik menggunakan piranti lunak dan Tabel 2×2. Kepekaan dan kekhasan demam, bertempat tinggal atau ditemukan riwayat perjalanan di daerah endemis malaria serta hitung monosit >8% dan hitung trombosit <150.000/mm³ dibandingkan pemeriksaan mikroskopis pada penelitian ini berturut-turut adalah 81,6% dan 81,8%. Nilai duga positif, nilai duga negatif, rasio kemungkinan positif dan rasio kemungkinan negatif pada penelitian ini berturut-turut adalah 88,6%, 72%, 4,5 dan 0,2. Penelitian ini mendapatkan kepekaan dan kekhasan demam, bertempat tinggal atau ditemukan riwayat perjalanan di daerah endemis malaria serta hitung monosit >8% dan hitung trombosit <150.000/mm³ yang tinggi dibandingkan pemeriksaan mikroskopis di pasien malaria.

Kata kunci: Malaria, tolok ukur hematologi, uji diagnostik

ABSTRACT

Malaria is still a major health problem in Tropical and Subtropical Countries particularly Asia and Afrika with a high morbidity dan mortality. Finding clinical symptoms such as fever after parasites which enter the blood, also clinical symptoms of hematological changes such as monocytosis and thrombocytopenia. This study was aimed to evaluate the diagnostic test of hematological parameters in patients suspect of malaria. This was a cross sectional diagnostic study conducted on 60 patients suspected of malaria with fullfilled inclusion and exclusion criteria from July 2015 to March 2016, at the Central Laboratory Dr. M. Djamil Hospital Padang, Reksodiwiryo Hospital Padang, Barung Belantai Puskesmas Pesisir Selatan, Hanafie Hospital Bungo, Sultan Thaha Saifuddin Hospital and Rimbo Bujang Puskesmas Tebo Hematological parameters such as monocytes and thrombocytes and also parasite microscopy were examined. Data was analysed by statistical software and diagnostic 2×2 table test. Sensitivity dan specificity of fever, stay in or history travelling to endemic malaria areas also result of monocyte count >8% and thrombocyte count <150,000/mm³ compared to parasite microscopy in this study were 81.6% and 81.8% respectively. Positive probability test, negative probability test, positive likelihood ratio and negative likelihood ratio in this study were 88.6%, 72%, 4.5 and 0.2 respectively. Sensitivity and specificity of fever, stay in or with history travelling to endemic malaria areas also monocyte count >8% and thrombocyte count <150,000/mm³ compared to parasite microscopy in this study was higher than patients suspected of malaria.

Key words: Malaria, hematology parameter, diagnostic test

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INTRODUCTION

Malaria is still a major health problem in Tropical and Subtropical Countries particularly Asia and Afrika with a high morbidity dan mortality.¹ It is one of important causes of febrile illnesses in the world.² Malaria remains the most important human parasitic disease globally, causing over 170 million clinical cases annually.³

Malaria is an infectious disease caused by Plasmodium parasites transmitted through the bite of female Anopheles mosquitoes.⁴ The World Health Organization (WHO) estimated about 3.2 billion people are at risk being infected of malaria. About 198 million cases of malaria occurred globally in 2013 and the disease led to 584,000 deaths.⁵

In Indonesia, malaria still a major health problem with an incidence rate of 30 million in a year and the death rate of about 120,000 cases annually.⁶ Kepulauan Seribu, island of Bali, island of Java, island of Sumatera, province of Papua, West Papua, Maluku, East Nusa Tenggara are endemic areas of malaria in Indonesia. Annual Parasite Incidence (API) in 1,000 Indonesian people in 2013 the highest was Papua (42.65), West Papua (38.44) and East Nusa Tenggara (16.37) respectively. The incidence of parasites in 1,000 people in 2013 in the Province of Jambi and West Sumatera respectively were 1.11 and 0.26.⁷

The clinical symptoms of malaria such as fever, chills, sweating, muscle aches, joint pain, malaise, fatigue, gastrointestinal disturbances (nausea, vomiting and diarrhea), headache, back pain, myalgia, and cough.⁸ Typical symptoms of malaria are fever chills followed by fever and sweating recurring every day, two days or three days depending on the Plasmodium.⁷

A rapid and accurate diagnosis is necessary for the effective management of malaria.^{9,10} Laboratory and clinical findings of malaria often give results that are not specific.⁹ Resulting in difficulties in establishing the correct diagnosis, so that the provision of antimalarial drugs become incorrect.²

World Health Organization recommended microscopic examination and Rapid Diagnostic Test (RDT) in patients suspected of malaria such are febrile illnesses patients who live in endemic areas of malaria, or with a history of travel to endemic areas of malaria.⁵ Microscopic examination of malaria parasites require trained personnel, tools and good staining of preparation.^{10,11} Negative result at the first microscopic examination can not rule out the diagnosis of malaria, especially if the level of parasitemia is low, so it requires repeated testing at intervals of 8–12 hours.

Microscopic examination can detect 5–20 parasites/mL of blood or a rate of parasitemia of 0.0001%.¹¹

Finding clinical symptoms such as fever after parasites enter the bloods stream, parasites can also form hematological changes. Hematological changes were found in patients with malaria such as normocytic normochrome anemia and thrombocytopenia.¹² Muwonge *et al.*⁹ in Uganda reported an increased levels of monocytes to 61 malaria patients of about 10.89 (6.23). However, studies of Chandra & Chandra¹³ in India during August 2008 until August 2010 reported that thrombocytopenia (platelet count <150,000/mm³) was significant in acute malaria compared to controls with a sensitivity of 87.2% and a specificity of 65%. Mangrio *et al.*³ in Pakistan reported that thrombocytopenia (platelet count <150,000/mm³) was significantly in malaria 86 patients (68%) compared to controls.

Examination of hematologic parameters is easier and faster than microscopic examination. It made the researchers interested in knowing the clinical diagnostic value of fever in patients who lived in endemic areas or with a history of travel to malaria-endemic areas, with changes in hematological parameters such as monocytes and platelet count compared to microscopic examination as the gold standard for malaria diagnosis.

METHODS

This cross sectional diagnostic study was conducted at the Central Laboratory Dr. M. Djamil Hospital Padang, Reksodiwiry Hospital Padang, Barung Belantai Puskesmas Pesisir Selatan, Hanafie Hospital Bungo, Sultan Thaha Saifuddin Hospital and Rimbo Bujang Puskesmas Tebo from July 2015 until March 2016.

Subjects of this study was all suspected malaria patients who had been diagnosed by clinicians, delivered and laboratory examination into the Central Laboratory Dr. M. Djamil Hospital Padang. Exclusion criteria were febrile illnesses in patients who had been diagnosed with systemic infections, typhoid fever, dengue fever and meningitis by the clinicians. Material for inspection of monocyte and platelet count and also microscopic examination of malaria was venous blood with EDTA anticoagulant. Preparation of thin and thick blood and Giemsa staining for microscopic examination of malaria were done in this study. Venous blood samples used for examination of monocyte and platelet count was stable for 24 hours at a temperature 2–8°C.

Data were collected and analyzed using a software. Univariate analysis used the data characteristics of the study subjects. The bivariate analysis carried out for a diagnostic test using Table 2×2 towards clinical fever residing in or travel history to malaria endemic areas as well as monocytes and platelets counts compared to microscopic examination to obtain the value of the sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio and the ratio of negative possibilities.

RESULTS AND DISCUSSION

This study was conducted on 60 patients with suspected malaria consisting of 38 subjects (63.3%) of positive malaria by microscopic examination, the males and females ratio was 14:1. The increase in malaria incidence ratio of males to females was different from the research conducted by Jairajpuri *et al.*² who stated that the ratio was 2,3:1. The increasing incidence of malaria in males than females was due to males more often traveled to malaria-endemic areas. This type of work often done by males like gardening also contributed to the high incidence of malaria in males.¹³

Fever is a common clinical symptom in 59 malaria patients (98.3%) with a mean temperature of 38.6°C (1.3). This result was not much different from the research reported by Saha *et al.*¹⁴ who revealed that an average Infection was the most cause of the fever, 40%, followed by neoplasms (20%), collagen diseases (20%), other diseases (10%) and unknown (10%). Fever in malaria infections was an intermittent fever which was the state of body temperature above normal but can also be found in body temperature dropping to a normal temperature for a few hours in one day.¹⁵ The classical symptoms of fever chills in malaria is often not visible in the initial infection. Not finding of symptoms of intermittent fever in malaria has not been able to exclude diagnosis of malaria.¹⁶ The basic subjects characteristics of this study is shown in Table 1.

Subjects in this study came from two areas in West Sumatra province and five districts in Jambi province. Jambi province is an endemic malaria.⁷ Based on the data of prevention and eradication of infectious diseases (P2PL) District of Tebo in 2014 reported that the number of malaria patients in Tebo regency was as many as 1803 people, with most cases coming from districts Rimbo Bujang II as many as 1,144 people.¹⁹

Table 1. The basic subjects characteristics

Characteristics	f (%)	Range	Mean (SD)
Gender			
– Males	45 (75)		
– Females	15 (25)		
Age (year)		9-75	32.9 (12.1)
– Children	4 (6.7)		
– Adult	56 (93.3)		
Clinical symptoms			
– Fever	59 (98.3)		
– Chills	40 (66.7)		
– Nausea	43 (71.7)		
– Vomiting	42 (70)		
– Headache	31 (51.7)		
– Abdominal pain	33 (55)		
– Joint pain	10 (16.7)		
– Malaise	9 (15)		
– Diarrhea	5 (8.3)		
– Cough	4 (6.7)		
– Pale	2 (3.3)		
– Bitter tongue	2 (3.3)		
Area of domicile			
– Padang City	33 (55)		
– District of Rimbo Bujang	11 (18.3)		
– District of Tebo	7 (11.6)		
– District of Bungo	6 (10)		
– District of South Pesisir	3 (5)		
History of travel to malaria-endemic areas			
– Papua	8 (13.3)		
– East Nusa Tenggara	7 (11.6)		

Note: f: frequency, SD: Standard Deviation

Researchers chose Jambi province rather than the Province of West Sumatra in taking the research subjects because; Jambi Province is an endemic malaria area based on the data of Ministry of Health⁷; easy transport from Jambi Province to the city of Padang where hematological parameters were done; the time required to transport was less than 24 hours so that the stability of the samples could be maintained.

Subjects in this study who did not reside in malaria-endemic areas such as the city of Padang, had a history of travel to malaria-endemic areas of Papua as many as eight people (13.3%) and Atambua, East Nusa Tenggara of 7 people (11.6%). This was in accordance with the data of Ministry of Health Republic of Indonesia in 2014 that the annual parasite incidence in 1000 people in Indonesia in 2013 was the highest in Papua (42.65), West Papua (38.44) and East Nusa Tenggara (16.37). The island of Sumatra, including West Sumatra province and Jambi province is the target region of malaria elimination in Indonesia in 2020 by decree of the Minister of Health Republic Indonesia in 2009 Number 293/MENKES/SK/IV/2009.⁷

This study showed an increase in monocyte count in malaria patients with a mean of 9.4 (5.4)%. This result was similar to Muwonge *et al.*⁹ who showed that an average count of monocytes in patients with malaria amounted to 10.89 (6.23)%. Monocytosis was one of hematological changes consistent in malaria.⁹ Monocytosis in malaria patients was associated with hyperplasia retikuloendotelial system.^{9,17} Monocyte changes in malaria depend on several factors such as acute infections, parasitaemia, severity of disease, the immune status of the host against malaria and concomitant infections.⁹

The mean platelet count in this study was found in the normal range as much as of 174,166.7 (1.1)/mm³, with the lowest value of 34,000/mm³ and the highest 410,000/mm³. Different results were obtained by Muwonge *et al.*⁹ and Jairajpuri *et al.*² and Chandra & Chandra.¹³ Study of Muwonge *et al.*⁹ reported thrombocytopenia in malaria patients with a mean platelet count of 99,490 (61,800)/mm³ in patients infected with *Plasmodium vivax* and 91,871 (59,900)/mm³ in patients infected with *Plasmodium falciparum*. Jairajpuri *et al.*² reported the mean platelet count in patients with malaria amounted to 90,000 (42,000)/mm³. Chandra & Chandra¹³ reported that the mean platelet count in patients with malaria amounted to 89,540 (85,360)/mm³.

The pathophysiology of thrombocytopenia in malaria is multifactorial¹⁴ sequestration of parasites and infected erythrocytes causing obstruction of the blood vessels, this leads to obstruction of activated

platelets. The release of Adenosine Diphosphate (ADP) during hemolysis of erythrocytes infected with the parasites will also lead to activated platelets. The number of activated platelets were unused due to a decline of platelet in the circulation.^{14,18}

This research showed that the mean platelet counts was within the normal range. This is likely caused by parasite immune complexes and erythrocytes that cause the destruction of platelets in the circulation while the endoplasmic reticulum is still mild so it does not cause a significant decrease in platelets in the circulation. The destruction of platelets in the circulation and the endoplasmic reticulum, which is heavy and the increased platelet activation due to obstruction of the blood vessels largely caused by *Plasmodium falciparum*. This study most of the malaria parasites was *Plasmodium vivax* (82.5%).

The ability of bone marrow to increase thrombopoiesis can also affect the platelet count in the circulation in malaria patients.¹⁸ This study showed the mean platelet counts within the normal range. This is likely due to the ability of bone marrow to increase the response thrombopoiesis destruction of platelets in the circulation while the endoplasmic reticulum was still good, no decreased platelet count in circulation in malaria patients in this study was found.

Distribution of *Plasmodium* of the malaria parasite in this study showed that the most was *Plasmodium vivax* in 37 subjects (97.3%) and *Plasmodium falciparum* in one subject (2.7%). An increase in the proportion of *Plasmodium vivax* in this study corresponded to the data of Ministry of Health ⁷ that *Plasmodium vivax* is the higher species that infect in Tropical Asia. Domicile area of subjects in this study were mostly from urban areas also affected the proportion of *Plasmodium vivax* in this study. The proportion of malaria based on the location of residence, according to Riskesdas data in 2013 found that infection with *Plasmodium vivax* (0.5%) in urban areas was higher than *Plasmodium falciparum* infection (0.3%).⁷

The diagnostic value of fever, residence or travel history in malaria endemic areas as well as the count of monocytes >8% and a platelet count <150,000/mm³ against microscopic examination of malaria based on Table 2×2 in this study obtained high a sensitivity and specificity were 81.6% and 81.8%, respectively, Positive Predictive Value (PPV) are 0.886 (88.6%) and Negative Predictive Value (NPV) are 0.72 (72%) and Positive Likelihood Ratio value (PLR) are 4.5 and a Negative Likelihood Ratio (NLR) were 0.2.

The sensitivity, specificity, NPV, as well as the PLR and NLR against fever, residence or travel history in malaria endemic areas as well as monocyte and

platelet count compared to microscopic examination in this study had a good diagnostic value. It can be attributed to that most malaria patients in the study showed symptoms of fever, reside in endemic areas or had a history of travel to malaria-endemic areas and increased monocytes count (monocytes >8%) and platelet counts decreased (platelet count <150,000/mm³).

This study has several limitations such as the researchers did not obtain data about of usage of antimalarial drugs before the hematologic and microscopic malaria examination in the study subjects. Researchers did not relate directly to the study subjects and obtained clinical data based on the patient's medical record. The use of antimalarial drugs can reduce and eliminate the malaria parasite immediately in the circulation, while improving hematological parameters so that monocytes and platelet count returned to normal range within a few days to once a week.

Molecular examination such as PCR was not done in this study because it was more expensive. Microscopy examination can detect 5–20 parasites/ μ L in blood.¹¹ Molecular examination have a better sensitivity and specificity than microscopy examination.^{8,9} This caused that molecular examination can detect lower parasitemia than microscopy examination.¹¹

CONCLUSION AND SUGGESTION

This study showed the sensitivity, specificity, NPV, as well as the PLR and NLR higher against fever, resided or travel history in malaria endemic areas as well as monocyte count >8% and a platelet count <150,000/mm³ higher than microscopic examination in malaria patients.

Therefore, we suggest the importance of obtaining data of antimalarial drugs usage before the hematologic and microscopic malaria examination in the subjects and further study is needed to use the gold standard for molecular examinations that can detect parasitemia level lower than microscopic examination.

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