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Research Article

Platelet Count and Platelet Indices Used as Potential Markers for First Malaria Infection Diagnosis

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ABSTRACT

Hematological changes, especially thrombocytopenia associated with malaria infection are well recognized. We aim to determine platelet count and platelet indices as risk markers for first screening of malaria infection diagnosis. A total of 100 patients with malaria infection attended at the Clinical Laboratory Unit, Thong Pha Phum Hospital. After parasitological confirmation of exclusive infection by malaria, platelet counts and platelet indices were determined. Receiver operating characteristic (ROC) analysis is used for calculation of the area under the curve (AUC) and estimation the sensitivity and specificity of these markers. Platelet count, mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT) were significantly altered in patients with malaria infection. A significant inverse relationship was observed between PCT and parasitemia (%Malaria). Platelet count is the highest AUC while the combination of platelet counts and PCT is the best risk marker for first malaria infection diagnosis with 99.0% sensitivity and 95% specificity, respectively. Therefore, platelet count and PCT indices are the effectiveness markers for malaria infection and may using for severity (%Malaria) demonstration in patients with malaria infection, especially in endemic area.

Keywords: Malaria infection, platelet count, platelet indices, risk markers

INTRODUCTION

Malaria is one of the most important parasitic infectious diseases in the world. It is a major health problem in the tropics with high morbidity and mortality[1]. The world malaria report in 2012, half of the world's population is at risk of malaria and an estimated 207 million cases led to nearly 627,000 deaths in 2012. *Plasmodium (P.) falciparum and P. vivax* are the most common. *P. falciparum* is the most deadly[2]. Several clinical complications have been described in malaria infection, including severe anemia, cerebral malaria, acute pulmonary edema, and multi-organ failure[3]. Although thrombocytopenia is often occurred, but bleeding is rare in these patients[4]. Hematological changes associated with malaria are well recognized, but the specific changes may vary with demographic factors, nutritional status, hemoglobinopathy, background, malaria endemicity levels, and malaria immunity[5]. Changes in platelet counts during acute malaria were reported in the several medical literatures, such as *P. falciparum* infections; these changes are the major cause of serious and complicated disease[6,7]. Many studies have also report the association of thrombocytopenia with *P. vivax* infection[8-10]. Peripheral destruction, excessive sequestration of platelets in spleen and excessive use of platelets associated with the disseminated intravascular coagulation phenomenon are underlying mechanisms of thrombocytopenia in malaria infection[11]. Addition with reduction in platelet (Plt) count and platelet function, changes in the volume and other features of platelet cells are the generally evidenced in these patients[12]. Moreover, platelets play an important role in the inflammatory response[13], enhanced disease severity and mortality during bacterial infections[14]. Furthermore, platelet activation alters the morphology change of platelets, included mean platelet volume (MPV), platelet distribution width (PDW)[15] and plateletcrit (PCT), which is a reliable measurement of platelet biomass[16]. All of these indices are considered as markers of platelet activation[11] and alteration in different clinical conditions[17,18]. Many studies reported the reduction of platelet count and alteration of platelet indices in patient with malaria infection[19,20]. Furthermore, this finding for the diagnosis of malaria infection can be perform to test for all suspected cases in all clinical laboratory. However, the association between these indices and the clinical outcome may useful in patients with malaria infection. The present study aimed to determine the alterations in platelet count, platelet indices for first screening in patients with malaria infection and tried to identify these platelets count and platelet indices as the risk markers for first screening of malaria infection.

MATERIALS AND METHODS

Subjects

This is a cross-sectional descriptive study based on the clinical and laboratory data of 100 patients with acute

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malaria infection and 100 normal subjects with non-malaria infection, who attended at Clinical Laboratory Unit, Thong Pha Phum Hospital between July 2014 – September 2014. All patient identifications with malaria infection were eligible to participate in this study. Our study was approved by the institutional ethical committee of Naresuan University, Phitsanulok, Thailand. After informed consent was obtained, all patients underwent a detailed clinical evaluation by our physician, including number of previous malaria infections and time of symptom onset at the time of patient's admission. One hundred normal subjects participated in the present study had no clinical history and/or finding of chronic liver disease, bleeding disorder, thrombocytopenia, drug intake or conditions that effect in blood changes were included in the study. All EDTA-anticoagulated blood samples were obtained to perform complete blood hemogram analysis, all laboratory testing were performed at the Clinical Laboratory Unit, Thong Pha Phum Hospital, Thong Pha Phum District, Kanchanaburi Province.

Hematological analysis and malaria diagnosis

Complete blood counts (CBC), platelet count and platelet indices were determined using the BC -5180 Auto Hematology analyzer (Mindray, Guangzhou city, Guangdong, China), which also provides results of MPV, PDW, and PCT. The normal ranges for MPV, PDW and PCT for this analyzer are as follow: Platelet count: 150.00 – 450.00 × 10^3 /µl; MPV: 7 -11 fL; PDW: 15.0 – 17.0 %; PCT: 0.108 – 0.282 %, respectively. Malaria was diagnosed on the basis of the microscopic examination of Giemsa-stained thick smears. Parasite densities were assessed as parasite/field. Minimum of 200 of oil immersion fields were assessed to label a negative smear if no parasites were detected. The grading of parasitemia (%Malaria) as percentage was done after counting of all malaria forms in oil immersion on thick and thin smears[21]. All slides were double-checked in a blinded manner.

Statistical Analysis

The Mann-Whitney non-parametric test was used to analyze the comparison the platelet counts and platelet indices between normal controls and patients with malaria infection. Spearman's correlation coefficients were calculated to evaluate the relationships of these markers including platelet count, platelet indices and parasitemia. We also calculated the area under the curve (AUC) of these markers and also calculated the sensitivity and specificity of these markers by using receiver operating characteristic (ROC) analysis. All analysis was performed by use of SPSS version 13.0 (SPSS, Chicago, IL, USA). The level of significance was set at *p*-value <0.05.

RESULTS

The patients had a median age of 24.5 years and interquartile 13.0-44.8 years, and 45 were males. The clinical characteristics were shown in Table 1. The diagnosis of malaria infection was diagnosed at the same day of admission in all patients. All patients had fever at the time of diagnosis. The median of % of malaria infection or parasitemia (%Malaria) level were 0.30%; interquartile 0.10-0.50%. No patient received the medications to interfere the platelet indices. The comparison of all platelet indices was significantly lower in patients with malaria infection than the normal controls ($p<0.05$) as in Table 2. The median (interquatile) of platelet count was 87.00 (62.00, 115.75) x 10^3 cells/µL; MPV was 9.100 (8.600, 9.900) fL; PDW was 16.20 (15.80-16.50) % and PCT was 0.0790 (0.0573-0.1078%). Bivariate correlation of these platelet indices demonstrated that platelet count was significantly correlated with MPV, PDW, PCT and %Malaria ($p<0.05$) and the others indices were demonstrated in the Table 3. In the present study, we used the ROC curve for calculated the area under curve (AUC) of the platelet count, PCT, PDW and MPV parameters, were 0.985 [95% confidence interval (CI) 0.971-0.999], 0.970 (95%CI 0.950-0.991), 0.876 (95%CI 0.828-0.924) and 0.843 (95%CI 0.789-0.897), respectively (Figure 1). We also compare the effectiveness of these platelet indices as the risk factors for screening strategies. We calculated the cut-off values of each platelet indices, Plt count was demonstrated the highest sensitivity and specificity and the others were demonstrated in Table 4. After we used the combination of platelet count and PCT [cutoff <150.00 x 10^3 (150,000) cell/µL, <0.119%], we found that the combination of platelet count and PCT was the best risk marker for first diagnosis of malaria infection with 99.0% sensitivity and 95% specificity (Table 4). Only one malaria infection patient received RBC and platelet transfusion during the admission in the hospital in this present study.

Table 1: Clinical and laboratory characteristics of all patients with malaria infection

Characteristics		n	%
Age (years) (n=100)	0-10	17	17
	11-20	25	25
	21-30	12	12
	31-40	15	15
	41-50	10	10
	51-60	11	11
	61-70	8	8
	71-80	2	2
Sex	Male	44	44
	Female	56	56
Fever at diagnosis (n=100)	Yes	100	100
Type of malaria infection	P.f. & P.v.	46	46
	P.f.	36	36
	P.v.	18	18
Throbocytopenia (/µL)	<50,000	13	13
	50,000-99,999	49	49
	100,000-149,999	33	33
	>150,000	5	5

Only one patients who received platelet and RBC transfusions

P.f.= *Plasmodium falciparum*; P.v.= *Plasmodium vivax*

DISCUSSION

Table 2: Comparison of platelet count and platelet indices in patients with malaria infection

Variables	Normal (n=100)	Malaria infection patients (n=100)	*p*-value
Age (years)	22.5(13.0-45.8)*	24.5(13.0-44.8)*	0.837
Plt count (/μL)	256.00 (203.00-352.25)	87.000(62.000-115.750)	<0.001
MPV (fL)	8.00 (7.5250-8.5000)	9.100 (8.600-9.900)	<0.001
PDW (%)	15.0500 (14.6250-15.6000)	16.200(15.800-16.500)	<0.001
PCT (%)	0.1705 (0.1383-0.2050)	0.0790 (0.0573-0.1078)	<0.001
%Malaria	-	0.30(0.10-0.50)	-

* median and interquartile range

Table 3: Bivariate correlation of platelet indices in patients with malaria infection

Correlation between parameters		Correlation coefficient	
		r	*p*-value
Plt count	MPV	-0.315	<0.001
	PDW	-0.373	<0.001
	PCT	0.912	<0.001
	%Malaria	-0.259	0.009
MPV	PDW	0.487	<0.001
PDW	PCT	-0.301	0.002
PCT	%Malaria	-0.279	0.005

Table 4: The effectiveness of platelet count and platelet indices cutoff levels as the risk factors for screening in patients with malaria infection

Parameters	Cutoff-Value	Sensitivity (%)	Specificity (%)
Plt count (/μL)	150,000	97	96
PCT (%)	0.119	94	90
MPV (fL)	8.55	78	79
PDW (%)	15.75	79	80
Plt count (/μL)+ PCT (%)	150,000+ 0.119	99.0	95

Malaria is the forest-related disease that high prevalent along the borders of Thailand. *Plasmodium falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi* are the common malaria parasites in Thailand. Thailand malaria report in 2014, estimated 29,317 patients of malaria infection, 56.8% were *P. vivax*, 42.5% were *P. falciparum*, remaining was the others[22]. All of these patients, 55% were Thai and 45% were foreign people. In the present study, the analysis of the platelet counts and platelet indices in patient with malaria infection reveals the high frequency of thrombocytopenia and alters in MPV, PDW and PCT. Platelet abnormalities in patients with malaria infection are both quantitative and qualitative, Platelet counts and PCT were significantly reduced in malarial infected patients in the present study. Thrombocytopenia is the common feature in both *P. falciparum* and *P. vivax* malaria infection, the incidence varies from 60%-80%[23], and it is more severe in complicated falciparum infection. Thrombocytopenia alone rarely causes bleeding unless it is accompanied by coagulopathy, which is observed only in severe complicated falciparum infection. Possible causes reduction of platelet survival from peripheral destruction (by immune, consumptive or other mechanisms), enhanced splenic uptake or sequestration and decreased platelet production. Many studies have been reported for the mechanisms of thrombocytopenia in malarial infection such as host produces oxidative stress and maintain it as a defense mechanism[24], reduction of erythrocytic anti-oxidative enzyme activities, and phagocytic cells release immune mediators and cytokines into the blood[25], immune complexes due to circulating immune binding to platelets or by absorption of soluble malaria antigen by platelets, and subsequent attachment of antibody to such antigens, disseminated intravascular coagulation (DIC). Other factors are defective platelet formation and hypersplenism[26]. Lower platelet counts and PCT were verified in patients with malaria infection in our present study. Previous studies, it has been demonstrated thrombocytopenia in malaria evolved from the increase peripheral platelets destruction rather than their insufficient production, this destruction is result from several immunologic events depending on antibody activities. Erel et al.[27] suggested that oxidative stress effect can be considered as reasons and mechanisms to cause thrombocytopenia. Platelet dysfunction during acute *P. falciparum* and *P. vivax* infection, hyperaggregation and enhanced platelet secretory activity were demonstrated[28]. In *in vitro* study demonstrated that normal platelets interact with the falciparum-infected erythrocytes and subsequently induce platelets hypersensitivity. The possibly mechanisms are stimulating through the ADP released from the infected red cells, antibody bound to platelets and the platelets invasion by malarial parasites. This present study demonstrated thrombocytopenia and altered platelet indices are the same as many recent studies[8,29]. Our study also demonstrated that the decreasing platelet count and PCT correlated with increasing levels of parasitemia (%Malaria). These platelet indices could be useful as risk marker for first malaria infection diagnosis and may be demonstrated the severity of the clinical approach in patients with malaria infection.

Limitation

The present study is a cross sectional, study in one hospital, only patients with *P. falciparum* and *P. vivax* malaria infection, short period and no more information of treatment and admission time. Further study may concentrate in the association of Plt count and PCT with the severity of malaria infection in the large population.

CONCLUSION

Platelet count and PCT parameters can use as the effectiveness markers for malaria infection and may demonstrate the clinical severity (%Malaria) in patients with malaria infection.

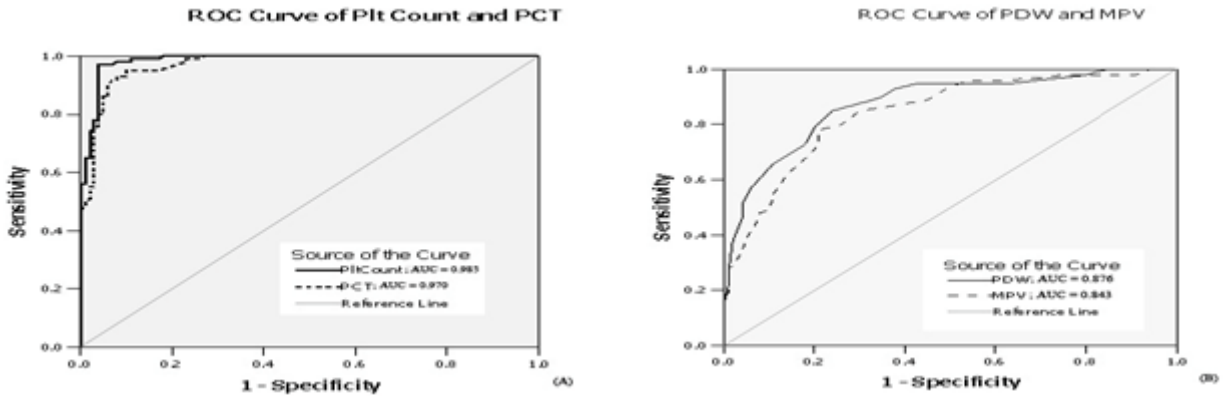

Figure 1: The AUC of (A) platelet count and PCT, (B) PDW and MPV, those were used as the risk factors for malaria screening

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