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The Hematological Alteration of Patients Parasitized by *Plasmodium vivax*

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Abstract

Malaria caused by *Plasmodium vivax* has placed huge burdens on the health, longevity, and general prosperity of large sections of the human population. In this study, the clinical profiles of patients infected with *P. vivax* in western Thailand were analyzed. A retrospective cross-sectional study of malaria cases resulting in hospitalization between 2013 and 2015 was collected. Clinical characteristics, diagnoses, and parasitological results on admission, age, and gender were mined from medical records at the laboratory unit, Phop Phra Hospital, located in endemic areas of Tak Province, Thailand. The results revealed that patients infected with *P. vivax* (276 cases) showed high monocyte counts during the initial stage of infection and continuously lower counts during the later stage of infection (p = 0.021). Low basophil counts during the initial stage of infection and continuously higher counts during the later stage of infection were also found (p = 0.033). Patients with many stages of *P. vivax* showed lower lymphocyte and basophil counts (p value = 0.011, 0.01), respectively. In conclusion, this study indicated the hematological alteration of patients infected with *P. vivax*. Alterations of monocyte and basophil counts during different stages of *P. vivax* infection were frequently found. In addition, many stages of *P. vivax* in the blood of patients were associated with lower lymphocyte counts. This information contributes to a better understanding of the pathological characteristics of *P. vivax* infection.

Keywords: *Plasmodium vivax*, asexual erythrocytic stages, hematological parameters

Introduction

Among the 4 species of malaria parasites that infect humans, *Plasmodium falciparum* and *Plasmodium vivax* vie for greatest prevalence in the world today. Although *P. falciparum* is justifiably regarded as the greater menace, because of the high levels of mortality with which it is associated, malaria caused by *P. vivax* has also placed huge burdens on the health, longevity, and general prosperity of large sections of the human population [1]. *P. vivax* is the most widely distributed human malaria parasite, with an at risk population of 2.5 billion. This parasite causes approximately 100 - 300 million clinical cases per year. Moreover, it has been reported that, due to chloroquine resistance in *P. vivax*, there is a lack of primaquine alternatives to attack the dormant liver-stage hypnozoites that can induce severe disease [2]. Severe manifestations in vivax malaria have also been reported, including cerebral malaria [3], hepatic dysfunction [4,5], renal dysfunction [6,7], severe anemia [5,8].

This study aimed to prospectively collect information on the clinical profile of malaria in subjects infected with *P. vivax* residing in some of the highest malaria transmission regions in Thailand. The results may add significantly to the prognostic value of a combination of simple clinical observations and readily available and affordable laboratory investigations.

Materials and methods

Ethical issue

This study protocol was reviewed and approved by the Phob Phra hospital and the Ethical Clearance Committee on Human Rights Related to Researches Involving Human Subjects of Walailak University (WU15/033). Inform consent forms were not obtained from patients due to the retrospective nature of the study.

Study design, study population, and data collection

This is a descriptive, retrospective study of malaria cases resulting in hospitalization between January 2013 and April 2015. The data were mined from medical records at Phop Phra hospital, located in endemic areas of Tak Province, Thailand. The instruments used included the following information: personal identification, clinical characteristics, diagnosis, and parasitological results on admission. After obtaining patient clinical data, including age and gender, venous blood samples were collected at the time of admission to the hospital to determine the presence of parasites and parasite count by thick and thin film examination. Complete blood counts (CBCs) by use of a BC-5200 Hematology Analyzer were also obtained (Mindray, Nanshan, Shenzhen, China). Those CBCs included red blood cell (RBC) counts, leukocyte counts, platelet counts, hemoglobin levels (Hbs), mean corpuscular volumes (MCVs), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentrations (MCHCs), and red cell distribution widths (RDWs). Asexual stages of *P. vivax* were counted into 5 categories, as diagnosed by laboratory protocol; ring form, amoeboid form, ring with amoeboid form, ring with amoeboid and schizont form, and any stage with gametocyte form found. In addition, to find out whether one or more than one stage of *P. vivax* could affect hematological parameters or not, asexual stages of parasite were defined into 2 categories; only one stage found, and more than one stage found.

Statistical analysis

Frequencies, central tendency, and dispersion were calculated. Tests for homogeneity of data were first performed. Tests for comparison of continuous variables in different groups were based on analysis of variance, Mann-Whitney U test, and Kruskal-Wallis tests up to distribution of data. Comparisons of proportions were based on Pearson chi-square tests. All p values were 2-tailed, with p value less than 0.05 considered statistically significant. Statistical analyses were performed using SPSS software, version 11.5 for Windows.

Results and discussion

There were 276 patients infected with *P. vivax* between January 2013 to April 2015 from thick and thin blood film examination. These patients were aged between 1 to 71 years old (mean 24.46 years). One hundred and forty-eight cases (53.6 %) were male, and 128 (46.4 %) were female. One hundred and sixty six (60.1 %) were Thai nationals, whereas 110 (39.9 %) were of other nationalities. There were no significant differences between the age, gender, of nationality of these patients with stage of *P. vivax* (p value > 0.05 by Kruskal Wallis test and Pearson chi-square test). After defining the group of patients according to the asexual stage of *P. vivax*, there were 2 cases with ring form (0.7 %), 107 cases with amoeboid form (38.7 %), 136 cases with ring/amoeboid form (49.3 %), 18 cases with ring/amoeboid/schizont (6.5 %), and 13 cases with any stage with gametocyte (4.8 %) (**Table 1**).

The results showed that patients who were infected with *P. vivax* (all 276 cases) had high monocyte counts (mean = 390 cells/μL) in the initial stage of infection and continuously lower counts in the later stage (any stage with gametocyte, mean = 230 cells/μL) of infection (p value = 0.021). This indicated that patients who were infected with *P. vivax* had high monocyte counts during the initial stage of infection, with continuously lower counts during the later stage of infection. Previous studies found that monocytosis was the most important leukocytic change associated with malaria infection, with increased counts being reported in malaria infection [9-11] and, correlating with this study, the results indicated 81.9 % cases with monocyte counts of more than 320/μL. High monocyte counts had been reported in

patients with uncomplicated malaria [12]. However, this was in contrast to a previous study, which reported low monocyte counts being associated with severe malaria and adverse outcomes [13]. Monocytes were activated by *Plasmodium* during first infection, and produced inflammatory cytokines, such as tumor necrosis factor (TNF), interleukin-1 (IL-1), and interleukin-6 (IL-6). These cytokines can stimulate the hepatic synthesis of acute phase inflammatory proteins, such as C-reactive protein (CRP), which increase during malaria infection. This is the cause of monocytosis during the initial stage of *P. vivax* infection. A previous study also demonstrated that these changes occur early in the disease, and some of the peripheral blood smears examined showed monocytes that contained malaria pigments and parasitized erythrocytes [10].

Table 1 General characteristics.

Demographic	Asexual stage of *Plasmodium vivax*					*p* value*
	Ring (n=2, 0.7%)	Amoeboid (n=107, 38.7%)	Ring/amoeboid (n=136, 49.3%)	Ring/amoeboid/schizont (n=18, 6.5%)	Any stage with gametocyte (n=13, 4.8%)	
Age, mean±SD	50.5±26.16	25.74±17.10	22.84±16.17	24.72±15.61	26.62±17.08	0.245*
Male/female, n (%)	1 (50)/1(50)	61 (57)/46 (43)	81 (59.6)/55 (40.4)	9 (50)/9 (50)	10 (76.9)/3 (23.1)	0.53**
Thai/Non-Thai, n (%)	2 (100)/ 0 (0)	62 (57.9)/ 45 (42.1)	69 (50.7)/67 (49.3)	13 (72.2)/5 (27.8)	6 (46.2)/7 (53.8)	0.497**

*Comparison of 5 groups using Kruskal Wallis test, **Comparison of 5 groups using Pearson chi-square test

Table 2 Hematological values in study population.

Hematological parameters	Asexual stage of *Plasmodium vivax* (mean±SD)					*p* value*
	Ring (n=2)	Amoeboid (n=107)	Ring/amoeboid (n=136)	Ring/amoeboid/schizont (n=18)	Any stage with gametocyte (n=13)	
WBC ($\times10^3$/μL)	5.91±2.75	6.28±2.37	6.27±2.10	6.14±2.32	5.99±1.75	0.982
Neutrophil ($\times10^3$/μL)	4.35±2.62	4.20±1.84	4.47±1.99	4.56±2.05	4.65±1.77	0.835
Lymphocyte ($\times10^3$/μL)	0.99±0.05	1.36±0.80	1.21±0.93	1.17±0.75	0.90±0.24	0.131
Monocyte ($\times10^3$/μL)	0.39±0.00	0.39±0.29	0.37±0.34	0.19±0.21	0.23±0.19	0.021
Eosinophil ($\times10^3$/μL)	0.16±0.11	0.25±1.08	0.16±0.17	0.16±0.13	0.14±0.09	0.824
Basophil ($\times10^3$/μL)	0.02±0.03	0.08±0.06	0.06±0.05	0.05±0.06	0.07±0.05	0.033
RBC ($\times10^6$/μL)	5.21±0.40	4.63±0.80	4.61±0.62	4.88±0.48	4.54±0.48	0.222
Hemoglobin (g/dL)	13.1±0.57	12.33±1.96	11.97±1.86	12.58±1.48	12.66±1.52	0.290
Hematocrit (%)	39±1.41	36.51±5.78	35.69±5.24	37.72±4.11	36.77±4.07	0.268
MCV (fL)	74.5±2.83	79.03±8.24	77.59±8.22	77.93±10.31	81±5.52	0.448
MCH (pg/cell)	25.2±0.85	26.93±2.70	26.13±3.20	25.98±3.73	27.97±2.79	0.183
MCHC (g/dL)	33.8±0.14	33.77±1.54	33.61±1.20	33.33±1.19	34.4±1.4	0.076
RDW (%)	13	12.50±1.20	12.73±1.50	12.78±0.75	12.61±1.2	0.252
Platelet ($\times10^3$/μL)	133±69.30	86.03±47.20	95.45±53.72	74.89±38.93	76.38±40.33	0.229

*Comparison of 5 groups using Kruskal Wallis test.

Table 3 Frequency of abnormal hematological values in study population.

Hematological parameters	Asexual stage of *Plasmodium vivax* (mean±SD)					Total (n=276)	*p* value*
	Ring (n=2)	Amoeboid (n=107)	Ring/amoeboid (n=136)	Ring/amoeboid/schizont (n=18)	Any stage with gametocyte (n=13)		
WBC<4000 /μL	1 (50%)	12 (11.2%)	15 (11%)	4 (22.2%)	1 (7.7%)	33 (12%)	0.293
Neutrophil>2100/μL	1 (50%)	23 (21.5%)	24 (17.6%)	4 (22.2%)	1 (7.7)	53 (19.2%)	0.556
Lymphocyte <800/μL	0	32 (29.9%)	52 (38.2%)	8 (44.4%)	4 (30.8%)	96 (34.8%)	0.443
Monocyte >320/μL	2 (100%)	92 (86%)	111 (81.6%)	11 (61.1%)	10 (76.9%)	226 (81.9%)	0.13
Eosinophil >200/μL	0	6 (5.6%)	11 (8.1%)	0	0	17 (6.2%)	0.542
Basophil >40/μL	1 (50%)	15 (14%)	31 (22.8%)	7 (38.9%)	2 (15.4%)	56 (20.3%)	0.082
RBC <4×10^6/μL	0	19 (17.8%)	20 (14.7%)	1 (5.6%)	1 (7.7%)	41 (14.9%)	0.588
Hemoglobin <11g/dL	0	22 (20.6%)	38 (27.9%)	3 (16.7%)	2 (15.4%)	65 (23.6%)	0.469
Hematocrit <36%	0	41 (38.3%)	62 (45.6%)	5 (27.8%)	3 (23.1%)	111 (40.2%)	0.208
MCV>100fL	2 (100%)	55 (51.4%)	77 (56.6%)	11 (61.1%)	6 (46.2%)	151 (54.7%)	0.556
MCH>32pg/cell	2 (100%)	33 (30.8%)	52 (38.2%)	9 (50%)	2 (15.4%)	98 (35.5%)	0.06
MCHC>37g/dL	0	4 (3.7%)	3 (2.2%)	0	0	7 (2.5%)	0.819
RDW>14.5%	0	5 (4.7%)	14 (10.3%)	1 (5.6%)	1 (7.7%)	21 (7.6%)	0.561
Platelet<150×10^3/μL	1 (50%)	98 (91.6%)	117 (86%)	17 (94.4%)	13 (100%)	246 (89.1%)	0.121

*Comparison of 5 groups using Pearson chi-square test

Table 4 Asexual stage of *Plasmodium vivax* and hematological parameters.

Hematological parameters	Asexual stage of *Plasmodium vivax* (mean±SD)		*p* value*
	One stage (n=110)	More than 1 stages (n=166)	
WBC ($\times10^3$/μL)	6.27±2.35	6.24±2.1	0.896
Neutrophil ($\times10^3$/μL)	4.20±1.83	4.5±1.98	0.284
Lymphocyte ($\times10^3$/μL)	1.35±0.79	1.18±0.88	0.011
Monocyte ($\times10^3$/μL)	0.39±0.29	0.34±0.32	0.054
Eosinophil ($\times10^3$/μL)	0.25±1.06	0.16±0.16	0.79
Basophil ($\times10^3$/μL)	0.08±0.06	0.06±0.05	0.010
Basophil (%)	1.30	0.91	0.018
RBC ($\times10^6$/μL)	4.64±0.8	4.63±0.6	0.863
Hemoglobin (g/dL)	12.35±1.93	12.09±1.81	0.161
Hematocrit (%)	36.56±5.72	35.99±5.08	0.263
MCV (fL)	79.02±2.35	77.84±8.3	0.347
MCH (pg/cell)	26.94±1.83	26.23±3.24	0.185
MCHC (g/dL)	33.79±0.79	33.63±1.22	0.164
RDW (%)	12.51±0.29	12.73±1.42	0.212
Platelet ($\times10^3$/μL)	86.79±1.06	91.84±51.91	0.412

*Comparison of 2 groups using Mann-Whitney U test

Patients infected with *P. vivax* had low basophil counts (mean = 20 cells/μL) in the initial stage of infection and continuously higher counts in the later stage of infection (mean at stage with gametocyte = 70 cells/μL) (*p* value = 0.033) (**Table 2**). This indicated patients infected with *P. vivax* had low basophil counts during the initial stage of infection and continuously higher counts during the later stage of infection. A previous study indicated that *P. falciparum* species infections can induce eosinophilia [9], which may occur in *P. vivax*. The eosinophilia occurring during infection can be explained by *P. falciparum* and *P. vivax* causing extensive changes in bone marrow structure and function, but eosinophil precursors are usually abundant and overactive [12,13]. Another stimulus of eosinophilia could be immunological responses to the malaria infections. There was a positive association between levels of TNF and sIL-2R and both eosinophil cationic protein (ECP) and eosinophil protein X/eosinophil-derived neurotoxin (EPX) in malaria patients, indicating that inflammatory reactions or T cell activation may play a role in eosinophil induction during malaria infection [10].

Overall, there were 226 (81.9 %) cases with monocyte counts of more than 320/μL. There were 246 (89.1 %) cases with platelet counts of less than 150,000/μL. Platelet counts were lower in most of the infected cases (89.1 %). A previous study revealed that the prevalence of thrombocytopenia was similar amongst both infections of *P. vivax* and *P. falciparum*, but patients with severe falciparum malaria had significantly lower platelet counts compared to non-severe falciparum malarial patients [14]. Thrombocytopenia might be due to peripheral destruction [15]. Immune-mediated destruction of circulating platelets is another factor causing thrombocytopenia in malaria infections [16].

White blood cell counts and differential counts were not significantly changed during stage transition of *P. vivax* (*p* value > 0.05). Other data of frequencies are also shown (**Table 3**). However, neutrophil percentage was significant higher than 70 % in 154 (55.8 %) cases (*p* value = 0.023). This was due to the activation and function of neutrophils during acute infection of *P. vivax* malaria. Neutrophils were shown to be highly activated, presenting enhanced phagocytic activity as well as superoxide production. The process of neutrophil activation could involve phagocytosis of opsonized parasites [17].

Patients with more than one stage infection tend to have lower lymphocyte counts (mean=1180 cells/μL) than patients with only one stage infection (mean = 1350 cells/μL)(*p* value = 0.011), whereas patients with more than one stage infection tend to have lower basophil counts (mean = 60 cells/μL) than patients with only one stage infection (mean = 80 cells/μL) (*p* value = 0.01) (**Table 4**). Patients with more than one stage infection tend to have lower lymphocyte counts (mean = 1180 cells/μL) than patients with only one stage infection (mean = 1350 cells/μL)(*p* value = 0.011). Evidence suggests that activation and dysfunction of T cells and lymphopenia is caused during malaria infection [18]. A previous study indicated that the relatively large drop in peripheral lymphocyte numbers would be a non-specific effect, such as pooling in the enlarged spleens of patients [19], rather than a response by malaria-specific lymphocytes only. Others studies pointed that the increased propensity of lymphocytes in malaria patients might undergo spontaneous apoptosis in vitro [20,21], possibly induced by soluble Fas ligand (sFasL)-Fas interaction [22]. This study has several limitations. First, the ring stage presented with other stages of *P. vivax* could be mixed infection with *P. falciparum*. Second, there were no data regarding other infectious agents (virus and bacteria) affecting hematological parameters. Third, data for thalassemia traits in patients infected with *P. vivax* were not available. Thalassemia traits can affect red blood cell parameters, such as causing lower MCV, MCH, and hemoglobin levels. Fourth, data about a normal group to compare with the other 5 groups were not available.

Conclusions

This study indicated that patients infected with *P. vivax* had high monocyte counts during the initial stage of infection, with continuously lower counts during the later stage of infection. In addition, patients infected with *P. vivax* had low basophil counts during the initial stage of infection, with continuously higher counts during the later stage of infection. Patients with more than one stage infection tended to have lower lymphocyte counts than patients with only one stage infection, whereas patients with more than one stage infection tended to have lower basophil counts than patients with only one stage infection.

This information contributes to a better understanding of the pathological characteristics of *P. vivax* infection.

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