FACTORS ASSOCIATED WITH SEVERE *PLASMODIUM VIVAX* MALARIA: A 15-YEAR RETROSPECTIVE STUDY

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Abstract. There has been a shift in the predominant species causing malaria in Thailand from *Plasmodium falciparum* to *Plasmodium vivax* due to malaria elimination efforts. In this study we aimed to determine the proportion of adult patients with severe *P. vivax* malaria admitted to the Hospital for Tropical Diseases, Bangkok, Thailand, during 1 January 2009 - 31 May 2023 and to identify the clinical factors significantly associated with malaria severity, in order to inform current treatment of *P. vivax* malaria at the study hospital. Inclusion criteria for study subjects were being aged ≥18 years, being admitted to the study hospital during the study period and being infected with *P. vivax* malaria mono-infection. Exclusion criteria for study subjects were having a co-infection with another species of malaria or a coinfection with another infectious disease and having incomplete medical records. The minimum number of subjects calculated to be needed for the study was 752. We retrospectively reviewed the medical records of each subject. Subjects were classified as having severe malaria infection using 2023 World Health Organization criteria. We used logistic regression analysis to identify factors significantly associated with severity. A total of 551 subjects were included in the study, fewer than the minimum number calculated to be needed, since these were all the subjects that met the inclusion and exclusion criteria during the study period at the study hospital. Of these, 478 (86.8%) were male. The mean (±standard deviation (SD)) age of the study subjects was 30.8 (±11.6) (range: 18-76) years. Of the total of 551 subjects 78 (14.2%) had severe *P. vivax* malaria. Twenty-one factors were significantly associated with severe infection on univariate analysis, 7 of these were removed due to multicollinearity or clinical irrelevance. Therefore, 14 factors were included in multivariable analysis. After eliminating confounding factors, the following factors were

significantly positively associated with severe *P. vivax* malaria: glucose-6-phosphate dehydrogenase (G6PD) deficiency (adjusted odds ratio (aOR) = 5.68, 95% confidence interval (CI): 2.41-13.35, $p<0.001$), fever on admission (aOR = 2.28, 95%CI: 1.19-4.38, $p=0.013$), gametocytemia (aOR = 2.30, 95%CI: 1.11-4.78, $p=0.025$), severe anemia (hemoglobin <8 g/dl) (aOR = 9.30, 95%CI: 1.81-47.77, $p=0.008$), an albumin level <3 mg/dl (aOR = 3.66, 95%CI: 1.19-11.25, $p=0.023$) and an elevated serum aspartate aminotransferase (AST) level 1-3 times greater than the upper normal limit (aOR = 2.42, 95%CI: 1.26-4.66, $p=0.008$). The following factor was significantly negatively associated with severe infection: male gender (aOR = 0.36, 95%CI: 0.15-0.84, $p=0.018$). In summary, 14.2% of subjects had severe infection and the factors significantly positively associated with severe infection were: having G6PD deficiency, having a fever on admission, having gametocytemia, having severe anemia, having a low serum albumin level and having a high AST level. The factor significantly negatively associated with severe malaria was being male. We conclude, subjects with these factors and who do not yet have a severe infection on admission should be monitored more carefully for deterioration and managed accordingly. Further studies are need to determine if there are interventions that can prevent non-severe patients with these risk factors from deteriorating to severe infection.

Keywords: associated factors, severe malaria, *Plasmodium vivax*, Thailand

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INTRODUCTION

Malaria is a major public health problem worldwide. *Plasmodium falciparum* malaria accounts for the majority of patients with severe malaria (WHO, 2022). In 1985, the World Health Organization (WHO) issued the first criteria to diagnose severe malaria and they have continued to revise these criteria regularly (Anonymous, 1986; White, 2022; WHO, 2023). The WHO criteria for severe malaria focus primarily on *P. falciparum* malaria in hyperendemic areas, such as Africa and parts of Asia (Dondorp *et al*, 2005; Dondorp *et al*, 2008; Dondorp

et al, 2010). These WHO severe malaria criteria are used worldwide for the management of malaria. There are no specifically defined malaria parasite density threshold criteria for severe *Plasmodium vivax* (*P. vivax*) malaria (WHO, 2023).

Malaria elimination efforts in Thailand have resulted in a change in the predominant species causing malaria in Thailand from *P. falciparum* malaria to *P. vivax* malaria (Thimasarn *et al*, 1995; WHO, 2022). In 2022, 94.7% of reported malaria patients in Thailand had *P. vivax* as the etiology (Thai Ministry of Public Health, 2022). *P. vivax* may cause severe malaria and death (Baird, 2013; Kojom Foko *et al*, 2021; Kotepui *et al*, 2020; Rahimi *et al*, 2014).

The number of severe *P. vivax* cases and their associated factors has not been studied in Thailand (Kotepui *et al*, 2020). In this study we aimed to determine the proportion of adult patients with severe *P. vivax* malaria admitted to the Hospital for Tropical Diseases, Bangkok, Thailand, during 1 January 2009 - 31 May 2023 and to identify the clinical factors significantly associated with malaria severity, in order the inform current treatment for *P. vivax* malaria at the study hospital.

MATERIALS AND METHODS

Study site and subject selection

This study was conducted at the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, a tertiary care tropical medicine referral center affiliated with a medical college. The study period was 1 January 2009 - 31 May 2023. The inclusion criteria for study subjects were being aged ≥18 years and being admitted to the study hospital during the study period with a diagnosis of *P. vivax* malaria mono-infection confirmed by microscopy. Exclusion criteria were having a co-infection with another species of malaria or a coinfection with another infectious disease and having incomplete medical records. The minimum number of subjects calculated to be needed for the study was 752. The proportion of severe cases was estimated to be 8.6% based on a previous study of severe *P. vivax* malaria (Rahimi *et al*, 2014). The population size was estimated to be 1000 subjects. The confidence limit was set at 1% and we used a 95% confidence interval (CI).

Study subjects were classified as having either severe or non-severe *P. vivax* malaria using WHO criteria

(WHO, 2023).

Data collection

The medical records for all study subjects meeting inclusion and exclusion criteria were retrospectively reviewed and the following data recorded: subject gender, age, nationality, history of present illness, history of previous malaria infections, vital signs, physical examination findings, body weight and height, initial laboratory results, pregnancy status (for females), length of hospitalization, need for blood component transfusions, hemodialysis, respiratory support, care in the intensive care unit (ICU), development of a hospital acquired infection (HAI) and treatment outcome, including death.

We used 2023 WHO criteria to classify severe malaria as primary outcome (WHO, 2023). Potential factors associated with severe malaria were defined according to clinical justification and previously published criteria (Bayisa and Dufera, 2022; Namazzi *et al*, 2022; WHO, 1979). These factors included the patient's demographic, clinical manifestations and laboratory data on admission. Continuous variables were categorized for meaningful interpretations and non-linear associations. We recorded the body mass index (BMI) of each subject and classified them as being underweight (BMI <18.5 kg/m^2), normal weight (BMI 18.5- 22.99 kg/m^2), overweight (BMI 23- 24.99 kg/m^2), obese (BMI 25- 29.99 kg/m^2) or morbidly obese (BMI >30 kg/m^2) (WHO Expert Consultation, 2004). Hepatomegaly was defined as a liver span >10 cm or a liver palpable >3 finger breaths below the right costal margin. Splenomegaly was defined as finding dullness to percussion at the left costovertebral angle or having a palpable spleen.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 29 (IBM Corp, Armonk, NY). Descriptive statistics were used to summarize the demographic baseline characteristics and laboratory data. The proportion of subjects with severe *P. vivax* malaria was calculated as the number of study subjects with severe *P. vivax* malaria divided by the total number of study subjects. Categorical data are presented as numbers and percentages (%). Chi-square or Fisher's Exact tests were used to compare categorical data, where

appropriate. The magnitudes of associations are calculated using odds ratios (OR) and 95% confidence intervals (95%CI). Numerical data are classified using means with standard deviations (SD) or medians with ranges, where appropriate. Normal distribution was assessed, in cases of uncertainty, using histograms and the Kolmogorov-Smirnov test. The independent samples t-test and Mann-Whitney U test were used to compare differences between two groups, where appropriate. Factors with a *p*-value <0.1 on univariate analysis were included on multivariate analysis. Simple logistic regression analysis was used to determine crude odds ratios (cOR). Multiple logistic regression analysis was used to eliminate confounding factors and determine adjusted odds ratios (aOR). Factors with a large proportion of subjects with missing data or clinical irrelevance were omitted. Pearson's correlation analysis was used to identify multicollinearity between two clinically related variables; the factor with a lower *p*-value, was used to determine significant associations between the two factors. All tests for significance were two-sided and a *p*-value <0.05 on multivariate analysis was considered statistically significant.

Ethical considerations

All study subjects gave written or fingerprint consent to have their data used anonymously for future studies, including this study. The study was conducted with approval by the director of the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. The protocol was reviewed and approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Thailand (MUTM 2023-062-01).

RESULTS

A total of 1,821 adult patients with malaria infection were admitted to the study hospital during 1 January 2009 - 31 May 2023. Of these, 1,270 were excluded due to having a non-*P. vivax* infection, being aged <18 years, having a mixed malaria infection, having a co-infection with another infectious disease or having incomplete records. The most common co-infections were dengue (11 cases), scrub typhus (2 cases) and hookworm (2 cases). A total of 551 subjects were included in the study: 78 subjects (14.2%) had severe *P. vivax* malaria (Fig 1, Table 1).

Of the 551 study subjects, 478 (86.8%) were male; 3 of the 73 female subjects (4.1%) were

pregnant on admission; all of whom had non-severe *P. vivax* malaria infection. Forty-seven percent of subjects (*n*=259) were Burmese, 30.0% (*n*=165) were Thais, 13.0% (*n*=72) were Laotian, 6.5% (*n*=36) were Cambodian, 1.4% (*n*=8) were Indians, 1.1% (*n*=6) were Pakistanis, 0.2% (*n*=1) was Belgian, 0.2% (*n*=1) was Irish, 0.2% (*n*=1) was Dutch and 0.2% (*n*=1) was Ethiopian. 97.8% of subjects (*n*=539) were aged 18-60 years and 2.0% (*n*=11) were aged >60 years. 9.8% of subjects (*n*=54) had glucose-6-phosphate dehydrogenase (G6PD) deficiency. 51.0% (*n*=281) reported having had malaria previously; 16.7% (*n*=92) within the previous 3 months. 44.1% of subjects (*n*=243) had fever before admission for <4 days, 52.3% (*n*=288) had fever before admission for 4-14 days and 2.9% (*n*=16) had fever before admission for >2 weeks. 2.7% of subjects (*n*=15) had a history of bleeding prior to admission, specifically: petechiae, gum bleeding, hemoptysis, gastrointestinal tract

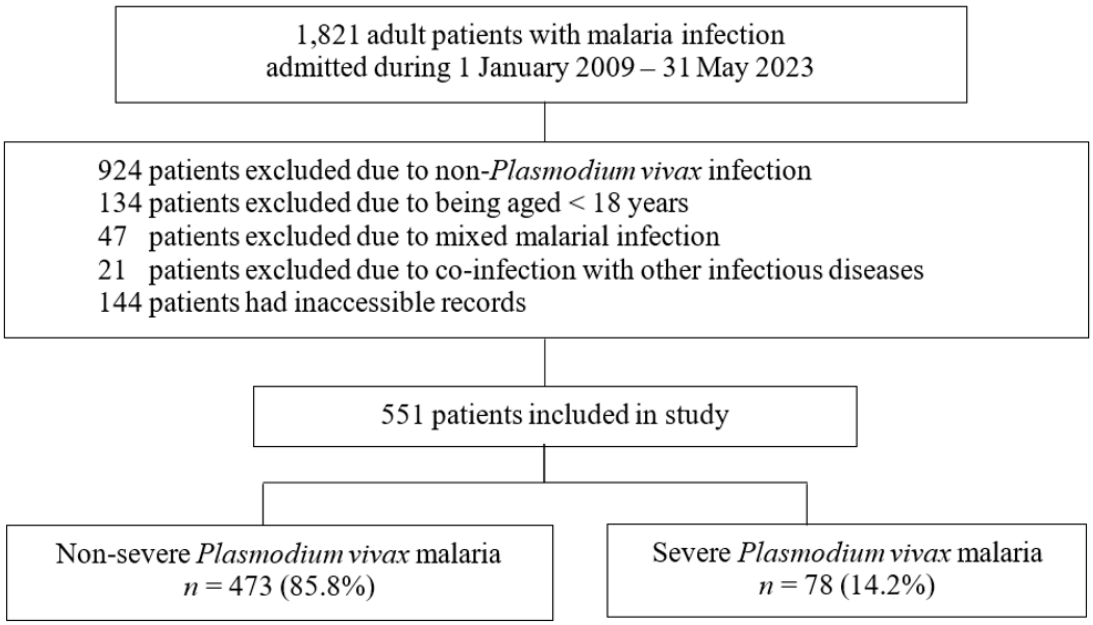

Fig 1 - Flow chart of patient selection and included cases

Table 1

Numbers of severe and non-severe *Plasmodium vivax* malaria subjects by year (N = 551)

Year	Numbers of non-severe *P. vivax* malaria cases	Numbers of severe *P. vivax* malaria cases	Percent of subjects with severe *P. vivax* malaria
2009	55	14	20.3
2010	40	4	9.1
2011	72	8	10.0
2012	83	6	6.7
2013	54	15	21.7
2014	53	6	10.2
2015	48	4	7.7
2016	13	3	18.8
2017	19	3	13.6
2018	10	4	28.6
2019	6	2	25.0
2020	2	4	66.7
2021	2	0	0.0
2022	15	2	11.8
2023	1	3	75.0
Summary	473	78	14.2

bleeding, hematuria or epistaxis (Table 2).

31.9% of study subjects (*n*=176) had fever (≥37.8oC) on admission (Table 3). The mean (±SD) heart rate was 88 (±17) beats/min and the mean (±SD) respiratory rate was 21 (±2.0) breaths/min. The mean (±SD) systolic blood pressure, diastolic blood pressure and mean arterial pressures were 113 (±15), 69 (±11) and 84 (±11) mmHg, respectively. 11.2% of study subjects (*n*=61) were underweight, 53.3% (*n*=294)

Table 2

Baseline characteristics and past medical history of study subjects on admission

Baseline characteristics	Non-severe *P. vivax* malaria subjects (N = 473)	Severe *P. vivax* malaria subjects (N = 78)	Total *P. vivax* malaria subjects (N = 551)	*p*-value*
Age groups, *n* (%)				0.076
18-25 years	203 (42.9)	34 (43.6)	237 (43.0)	
26-45 years	211 (44.6)	28 (35.9)	239 (43.4)	
46-60 years	51 (10.8)	12 (15.4)	63 (11.4)	
>60 years	7 (1.5)	4 (5.1)	11 (2.0)	
Missing data	1 (0.2)	0 (0)	1 (0.2)	
Gender, *n* (%)				0.006
Female	55 (11.6)	18 (23.1)	73 (13.2)	
Male	418 (88.4)	60 (76.9)	478 (86.8)	
Pregnancy status, *n* (%) (N=73)				
Not pregnant	52 (94.5)	18 (100)	70 (95.9)	N/A
Pregnant	3 (5.5)	0 (0)	3 (4.1)	

Table 2 (cont)

Baseline characteristics	Non-severe *P. vivax* malaria subjects (N = 473)	Severe *P. vivax* malaria subjects (N = 78)	Total *P. vivax* malaria subjects (N = 551)	*p*-value*
Nationality, *n* (%)				0.104
Thai	138 (29.2)	27 (34.6)	165 (30.0)	
Burmese	226 (47.8)	33 (42.3)	259 (47.0)	
Laotian	65 (13.7)	7 (9.0)	72 (13.0)	
Cambodian	31 (6.6)	5 (6.4)	36 (6.5)	
Others	12 (2.5)	6 (7.7)	18 (3.3)	
Not recorded	1 (0.2)	0 (0)	1 (0.2)	
G6PD deficiency, *n* (%)				<0.001
No	419 (88.6)	56 (71.8)	475 (86.2)	
Yes	38 (8.0)	16 (20.5)	54 (9.8)	
Not recorded	16 (3.4)	6 (7.7)	22 (4.0)	
Number of previous malaria infections, *n* (%)				0.858
0	114 (24.1)	21 (26.9)	135 (24.5)	
1	147 (31.1)	24 (30.9)	171 (31.0)	
2	44 (9.3)	6 (7.7)	50 (9.1)	
3	31 (6.5)	3 (3.8)	34 (6.2)	
≥4	23 (4.9)	3 (3.8)	26 (4.7)	
Not recorded	114 (24.1)	21 (26.9)	135 (24.5)	

Table 2 (cont)

Baseline characteristics	Non-severe *P. vivax* malaria subjects (N = 473)	Severe *P. vivax* malaria subjects (N = 78)	Total *P. vivax* malaria subjects (N = 551)	*p*-value*
Time since last malaria infection, *n* (%)				0.349
Never	114 (24.1)	21 (26.9)	135 (24.5)	
<3 months	79 (16.7)	13 (16.7)	92 (16.7)	
4-12 months	70 (14.8)	7 (9.0)	77 (14.0)	
>1 year	44 (9.3)	11 (14.1)	55 (10.0)	
Not recorded	166 (35.1)	26 (33.3)	192 (34.8)	
Duration of fever in days, *n* (%)				0.035
0-3	211 (44.6)	32 (41.0)	243 (44.1)	
4-7	212 (44.8)	31 (39.7)	243 (44.1)	
8-14	32 (6.8)	13 (16.7)	45 (8.2)	
≥15	14 (3.0)	2 (2.6)	16 (2.9)	
Not recorded	4 (0.8)	0 (0)	4 (0.7)	
Abnormal bleeding†, *n* (%)				0.047
No	463 (97.9)	73 (93.6)	536 (97.3)	
Yes	10 (2.1)	5 (6.4)	15 (2.7)	

*p-value <0.1 was considered statistically significance; †Abnormal bleeding are such as petechiae, gum bleeding, hemoptysis, gastrointestinal tract bleeding, hematuria or epistaxis, except a normal menstrual cycle in female

G6PD: glucose-6-phosphate dehydrogenase; *P. vivax*: *Plasmodium vivax*; N/A: not applicable or cannot be calculated

were normal weight, 10.5% (*n*=58) were overweight, 12.7% (*n*=70) were obese and 4.7% (*n*=26) were morbidly obese. 24.7% of subjects (*n*=136) had hepatomegaly and 13.8% (*n*=76) had splenomegaly on admission (Table 3).

56.3% of subjects (*n*=310) had an asexual malaria parasite density >10,000 /µl; 22.7% (*n*=125) had gametocytemia and 38.1% (*n*=210) had schizontemia. 2.7% of subjects (*n*=15) had severe anemia and 51.0% (*n*=281) had mild anemia. 60.4% of subjects (*n*=333) had a platelet count ≤100,000 cells/µl. 0.6% of subjects (*n*=4) had a high blood urea nitrogen (BUN) level (>40 mg/dl) and 0.5% (*n*=3) had a high creatinine level (>2mg/dl). 4.9 % of subjects (*n*=27) had a low bicarbonate level (≤20 mmol/l). 11.1% of subjects (*n*=61) had a high total bilirubin level (≥2.5 mg/dl), 6.7% (*n*=37) had a low albumin level (<3 mg/dl) and 1.8% (*n*=10) had a high aspartate aminotransferase (AST) level (>3 times the upper normal limit; >96 U/I). No study subjects had hypoglycemia (<40 mg/dl) (Table 4).

55.1% of subjects with severe *P. vivax* malaria (*n*=43) had jaundice, 29.5% (*n*=23) had respiratory distress, 21.8% (*n*=17) had shock, 7.7% (*n*=6) had bleeding, 7.7% (*n*=6) had severe anemia, 3.8% (*n*=3) had prostration, 2.6% (*n*=2) had impaired consciousness, 2.6% (*n*=2) had acute kidney injury, 1.3% (*n*=1) had multiple seizures, 1.3% (*n*=1) had metabolic acidosis, 1.3% (*n*=1) was intubated and placed on mechanical ventilation and 1.3% (*n*=1) had hemodialysis (7 times).

Out of the total of 551 subjects 0.7% (*n*=4) developed pneumonia during their hospitalization and 3.6% (*n*=20) were treated in the intensive care unit. All except 4 subjects were cured and discharged home; these 4 signed out against medical advice. No deaths occurred among study subjects (Table 5).

Univariate analysis

On univariate analysis, 21 variables were potentially associated with severe malaria ($p<0.1$): age groups ($p=0.076$), gender ($p=0.006$), G6PD deficiency ($p<0.001$), duration of fever ($p=0.035$), bleeding ($p=0.047$), fever on admission ($p=0.002$), heart rate ($p<0.001$), respiratory rate ($p=0.016$), gametocytemia ($p=0.033$), schizontemia ($p=0.037$), hemoglobin levels ($p<0.001$), hematocrit levels ($p<0.001$), platelet count ($p=0.024$), BUN ($p<0.001$), creatinine ($p=0.031$), bicarbonate levels ($p=0.008$), total bilirubin levels ($p<0.001$), albumin levels ($p<0.001$), aspartate aminotransferase levels ($p<0.001$),

Table 3

Examination findings of study subjects on admission

Physical examination findings on admission	Non-severe *P. vivax* malaria subjects (N = 473)	Severe *P. vivax* malaria subjects (N = 78)	Total *P. vivax* malaria subjects (N = 551)	*p*-value*
Temperature on admission, *n* (%)				0.002
<37.8°C	334 (70.6)	41 (52.6)	375 (68.1)	
≥37.8°C	139 (29.4)	37 (47.4)	176 (31.9)	
Heart rate (beats/min), mean±SD	86.9±17.2	96.7±19.1	88.3±17.8	<0.001
Respiratory rate (breaths/min), mean±SD	20.8±1.7	21.6±3.1	20.9±2.0	0.016
SBP (mmHg), mean±SD	113.2±14.0	111.8±21.2	112.9±15.2	0.590
DBP (mmHg), mean±SD	69.1±10.0	67.8±13.4	68.9±10.6	0.435
MAP (mmHg), mean±SD	83.8±10.3	82.5±14.7	83.5±11.0	0.462
BMI range, *n* (%)				0.167
Underweight (<18.5 kg/m^2)	56 (11.8)	5 (6.4)	61 (11.2)	
Normal (18.5-22.9 kg/m^2)	255 (53.9)	39 (50.0)	294 (53.3)	
Overweight (23.0-24.9 kg/m^2)	55 (11.6)	3 (3.9)	58 (10.5)	
Obesity (25.0-29.9 kg/m^2)	58 (12.3)	12 (15.4)	70 (12.7)	
Morbid obesity (≥30.0 kg/m^2)	21 (4.5)	5 (6.4)	26 (4.7)	
Weight or height not recorded	28 (5.9)	14 (17.9)	42 (7.6)	

Table 3 (cont)

Physical examination findings on admission	Non-severe *P. vivax* malaria subjects (N = 473)	Severe *P. vivax* malaria subjects (N = 78)	Total *P. vivax* malaria subjects (N = 551)	*p*-value*
Hepatomegaly, *n* (%)				0.108
No	360 (76.1)	53 (68.0)	413 (75.0)	
Yes	111 (23.5)	25 (32.0)	136 (24.7)	
Not recorded	2 (0.4)	0 (0)	2 (0.3)	
Splenomegaly, *n* (%)				0.137
No	410 (86.7)	63 (80.8)	473 (85.8)	
Yes	61 (12.9)	15 (19.2)	76 (13.8)	
Not recorded	2 (0.4)	0 (0)	2 (0.4)	

**p*-value <0.1 was considered statistically significance

BMI: body mass index; DBP: diastolic blood pressure; kg/m^2: kilogram per square meter; MAP: mean arterial pressure; min: minutes; mmHg: millimeters of mercury; N/A: not applicable or cannot be calculated; *P. vivax: Plasmodium vivax*; SBP: systolic blood pressure; SD: standard deviation; °C: degree Celsius

Table 4

Laboratory results of study subjects on admission

Laboratory results on admission	Non-severe *P. vivax* malaria subjects (N = 473)	Severe *P. vivax* malaria subjects (N = 78)	Total *P. vivax* malaria subjects (N = 551)	*p*-value*
Parasite count, *n* (%)				0.132
≤10,000 parasites/μl	213 (45.0)	28 (35.9)	241 (43.7)	
>10,000 parasites/μl	260 (55.0)	50 (64.1)	310 (56.3)	
Gametocytes, *n* (%)				0.033
Absent	373 (78.9)	53 (68.0)	426 (77.3)	
Present	100 (21.1)	25 (32.0)	125 (22.7)	
Schizonts, *n* (%)				0.037
Absent	301 (63.6)	40 (51.3)	341 (61.9)	
Present	172 (36.4)	38 (48.7)	210 (38.1)	
Hemoglobin, *n* (%)				<0.001
No anemia (>12 g/dl in females, >13 g/dl in males)	219 (46.3)	35 (44.9)	254 (46.1)	
Mild anemia (8-12 g/dl in females, 8-13 g/dl in males)	249 (52.6)	32 (41.0)	281 (51.0)	
Severe anemia (<8 g/dl)	4 (0.9)	11 (14.1)	15 (2.7)	
Test not done	1 (0.2)	0 (0)	1 (0.2)	

Table 4 (cont)

Laboratory results on admission	Non-severe *P. vivax* malaria subjects (N = 473)	Severe *P. vivax* malaria subjects (N = 78)	Total *P. vivax* malaria subjects (N = 551)	*p*-value*
Hematocrit, *n* (%)				<0.001
No anemia (>36% in females, >41 % in males)	146 (30.9)	14 (18.0)	160 (29.0)	
Mild anemia (25-35.99% in females, 25-40.99 % in males)	270 (57.1)	39 (50.0)	309 (56.1)	
Severe anemia (<25%)	2 (0.4)	7 (8.9)	9 (1.6)	
Test not done	55 (11.6)	18 (23.1)	73 (13.3)	
Platelet count, *n* (%)				0.024
>150,000 cells/µl	80 (16.9)	7 (8.9)	87 (15.8)	
100,001–150,000 cells/µl	117 (24.7)	13 (16.7)	130 (23.6)	
≤100,000 cells/µl	275 (58.1)	58 (74.4)	333 (60.4)	
Test not done	1 (0.3)	0 (0)	1 (0.2)	
Blood Urea Nitrogen, *n* (%)				<0.001
Normal (<20 mg/dl)	436 (92.2)	69 (88.5)	505 (91.7)	
Mild elevation (20-40 mg/dl)	14 (3.0)	4 (5.1)	18 (3.3)	
Abnormal (>40 mg/dl)	1 (0.2)	3 (3.9)	4 (0.6)	
Test not done	22 (4.6)	2 (2.5)	24 (4.4)	

Table 4 (cont)

Laboratory results on admission	Non-severe *P. vivax* malaria subjects (N = 473)	Severe *P. vivax* malaria subjects (N = 78)	Total *P. vivax* malaria subjects (N = 551)	*p*-value*
Creatinine, *n* (%)				0.031
Normal (<1.2 mg/dl)	404 (85.4)	65 (83.3)	469 (85.1)	
Mild elevation (1.2-2 mg/dl)	45 (9.5)	9 (11.5)	54 (9.8)	
Abnormal (>2 mg/dl)	1 (0.2)	2 (2.6)	3 (0.5)	
Test not done	23 (4.9)	2 (2.6)	25 (4.6)	
Bicarbonate, *n* (%)				0.008
>20 mmol/l	426 (90.1)	65 (83.3)	491 (89.1)	
≤20 mmol/l	18 (3.8)	9 (11.5)	27 (4.9)	
Test not done	29 (6.1)	4 (5.2)	33 (6.0)	
Total bilirubin, *n* (%)				<0.001
<2.5 mg/dl	427 (90.3)	32 (41.0)	459 (83.3)	
≥2.5 mg/dl	16 (3.4)	45 (57.7)	61 (11.1)	
Test not done	30 (6.3)	1 (1.3)	31 (5.6)	
Albumin, *n* (%)				<0.001
≥3 mg/dl	424 (89.6)	59 (75.6)	483 (87.7)	
<3 mg/dl	19 (4.0)	18 (23.1)	37 (6.7)	
Test not done	30 (6.4)	1 (1.3)	31 (5.6)	

Table 4 (cont)

Laboratory results on admission	Non-severe *P. vivax* malaria subjects (N = 473)	Severe *P. vivax* malaria subjects (N = 78)	Total *P. vivax* malaria subjects (N = 551)	*p*-value*
AST, *n* (%)				<0.001
Normal (0-32 U/l)	325 (68.7)	34 (43.6)	359 (65.2)	
1-3 times x UNL (33-96 U/l)	121 (25.6)	42 (53.8)	163 (29.6)	
>3 times x UNL (>96 U/l)	9 (1.9)	1 (1.3)	10 (1.8)	
Test not done	18 (3.8)	1 (1.3)	19 (3.4)	
ALT (U/l) (N = 532), median (range)	25 (4-212)	31 (5-200)	26 (4-212)	0.01
ALP (U/l) (N = 532), mean±SD	91.5±38.5	114.1±58.9	94.9±42.8	0.002
Serum glucose, *n* (%)				N/A
Serum glucose ≥40 mg/dl	269 (56.9)	51 (65.4)	320 (58.1)	
Hypoglycemia <40 mg/dl)	0 (0)	0 (0)	0 (0)	
Test not done	204 (43.1)	27 (34.6)	231 (41.9)	

*p-value <0.1 was considered statistically significance

ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; g/dl: gram per deciliter; mg/dl: milligram per deciliter; mmol/l: millimole per liter; µl: microliter; N/A: not applicable; *P. vivax: Plasmodium vivax*; SD: standard deviation; UNL: upper normal limit; U/l: units per liter

Table 5

Hospital outcomes of study subjects (N = 551)

Outcomes of study subjects	Results
Severity, *n* (%)	
Non-severe	473 (85.8)
Severe	78 (14.2)
Number of patients who met WHO criteria for severe malaria (WHO, 2023)	
Impaired consciousness (GCS <11)	2
Prostration	3
Multiple convulsions (≥2 episodes in 24 hours)	1
Acidosis (bicarbonate <15 mmol/l)	1
Severe anemia (hemoglobin <7 g/dl or hematocrit <20%)	6
Renal impairment (creatinine >3 mg/dl or BUN >56 mg/dl)	2
Jaundice (total bilirubin >3 mg/dl)	43
Respiratory distress (respiratory rate >30 breaths/min, evidence of pulmonary edema)	23
Significant bleeding	6
Shock (blood pressure <90/60 mmHg or MAP < 65mmHg)	17
Hypoglycemia (serum glucose <40 mg/dl)	0
Number of criteria indicating severe infection per subject, *n* (%)	
1 criterion	61 (78.2)
2 criteria	12 (15.4)
3 criteria	4 (5.2)
≥4 criteria	1 (1.2)
Median (range) length of hospital stay in days	5 (1-35)
Oxygen support, *n* (%)	
Not needed	532 (96.6)
Oxygen via cannula	18 (3.2)
Intubation	1 (0.2)

Table 5 (cont)

Outcomes of study subjects	Results
Outcome, *n* (%)	
Cured	547 (99.3)
Discharged against advice	4 (0.7)
Death	0 (0.0)
ICU transfer, *n* (%)	20 (3.6)
Blood component transfusion, *n* (%)	21 (3.8)
Hemodialysis, *n* (%)	1 (0.2)
Developed pneumonia during admission, *n* (%)	4 (0.7)

BUN: blood urea nitrogen; g/dl: gram per deciliter; GCS: Glasgow coma score; ICU: intensive care unit; MAP: mean arterial pressure; mg/dl: milligram per deciliter; min: minutes; mmHg: millimeters of mercury; mmol/l: millimole per liter; WHO: World Health Organization

alanine aminotransferase (p=0.01), alkaline phosphatase (p=0.002) (Tables 2, 3 and 4).

Five variables were removed from multivariate analysis due to multicollinearity: hematocrit, creatinine, total bilirubin, alanine aminotransferase and alkaline phosphatase. Although the heart and respiratory rates were significantly different between mild and severe malaria cases, the mean values were in the normal range so they were removed because they lacked clinical significance. Therefore, a total of 14 variables were included in the multivariate analysis.

Multivariate analysis

The 14 factors included in multivariate analysis were: subject age group, gender, history of G6PD deficiency, presence of fever on admission, duration of fever, presence of bleeding, gametocytemia, schizontemia, hemoglobin level, platelet level, BUN level, bicarbonate level, albumin level and AST level. Seven factors were found to be significantly associated with severe *P. vivax* malaria on multivariate analysis: male gender (adjusted odds ratio (aOR) = 0.36, 95% confidence interval (CI): 0.15-0.84, p=0.018), G6PD deficiency (aOR = 5.68,

95%CI: 2.41-13.35, p<0.001), fever on admission (aOR = 2.28, 95%CI: 1.19-4.38, p=0.013), gametocytemia (aOR = 2.30, 95%CI: 1.11-4.78, p=0.025), a hemoglobin level <8 g/dl (aOR = 9.30, 95%CI: 1.81-47.77, p=0.008), an albumin level <3 mg/dl (aOR = 3.66, 95%CI: 1.19-11.25, p=0.023) and an AST level 1-3 times the upper normal limit (aOR = 2.42, 95%CI: 1.26-4.66, p=0.008) (Table 6).

DISCUSSION

Proportion of subjects with severe malaria

In our study, 14.2% of subjects had severe *P. vivax* malaria, much higher than the 0.4% reported in a study from Tak Province, Thailand along the Thai Myanmar border (Luxemburger *et al*, 1997) and higher than 0.3% reported in a study from Trat and Chanthaburi Provinces, Thailand along the Thai Cambodian (Wangroongsarb *et al*, 2012). However, it should be noted that our hospital is a referral hospital for tropical diseases, so more severe cases are referred to our hospital while milder cases are treated at other hospitals, resulting in a higher proportion of our patients being sicker. There are no other studies of the proportion of *P. vivax* cases being severe in Thailand.

A study from northern Papua, Indonesia (Lusiyana *et al*, 2019) reported the proportion of severe *P. vivax* cases to be 9.3%. A study from Vietnam (Duong *et al*, 2023) reported the proportion of severe *P. vivax* cases to be 10.5%. Studies from Bikaner, Manipal and Kolkata, India, reported the proportions of severe *P. vivax* cases ranged from 16.9% to 56.8% (Acharya and Harwani, 2022; Kumar and Saravu, 2017; Saravu *et al*, 2014; Sarkar *et al*, 2013).

Factors associated with severe P. vivax malaria

In our study, male gender was significantly negatively associated with severe *P. vivax* malaria. The majority of our subjects were males. A previous meta-analysis of patients with severe *P. vivax* malaria, found females had a greater risk of death due to respiratory complications, including acute respiratory distress syndrome (Val *et al*, 2017). A study of patients with *P. vivax* malaria from Papua, Indonesia reported females were significantly more likely to have severe symptoms and need for hospitalization (Tjitra *et al*, 2008). Women with *P. vivax* malaria infection are at greater risk for severe malaria during pregnancy (Kumar and Saravu, 2017). In our study, however, none of the

Table 6

Logistic regression analysis of association between severe *Plasmodium vivax* malaria and selected variables

Characteristics of study patients	Non-severe *P. vivax* malaria subjects *n* (%)	Severe *P. vivax* malaria subjects *n* (%)	cOR (95%CI)	aOR (95% CI)
Age groups (N = 550)				
18-25 years	203 (85.7)	34 (14.3)	1	1
26-45 years	211 (88.3)	28 (11.7)	0.79 (0.46-1.35)	0.62 (0.30-1.27)
46-60 years	51 (81.0)	12 (19.0)	1.41 (0.68-2.90)	0.75 (0.28-2.01)
>60 years	7 (63.6)	4 (36.4)	3.41 (0.95-12.28)	1.72 (0.34-8.76)
Gender (N = 551)				
Female	55 (75.3)	18 (24.7)	1	1
Male	418 (87.5)	60 (12.5)	0.44 (0.24-0.80)	0.36 (0.15-0.84)
G6PD deficiency (N = 529)				
No	419 (88.2)	56 (11.8)	1	1
Yes	38 (70.4)	16 (29.6)	3.15 (1.65-6.02)	5.68 (2.41-13.35)
Duration of fever (N= 547)				
0-3 days	211 (86.8)	32 (13.2)	1	1
4-7 days	212 (87.2)	31 (12.8)	0.96 (0.57-1.64)	0.90 (0.45-1.82)
8-14 days	32 (71.1)	13 (28.9)	2.68 (1.27-5.64)	2.92 (0.99-8.57)
≥15 days	14 (87.5)	2 (12.5)	0.94 (0.20-4.34)	0.43 (0.04-4.81)

Table 6 (cont)

Characteristics of study patients	Non-severe *P. vivax* malaria subjects *n* (%)	Severe *P. vivax* malaria subjects *n* (%)	cOR (95%CI)	aOR (95% CI)
Abnormal bleeding (N = 551)				
No	463 (86.4)	73 (13.6)	1	1
Yes	10 (66.7)	5 (33.3)	3.17 (1.05-9.54)	4.08 (0.65-25.51)
Temperature on admission (N = 551)				
<37.8°C	334 (89.1)	41 (10.9)	1	1
≥37.8°C	139 (79.0)	37 (21.0)	2.17 (1.33-3.53)	2.28 (1.19-4.38)
Gametocytes (N = 551)				
Absent	373 (87.6)	53 (12.4)	1	1
Present	100 (80.0)	25 (20.0)	1.76 (1.04-2.97)	2.30 (1.11-4.78)
Schizonts (N = 551)				
Absent	301 (88.3)	40 (11.7)	1	1
Present	172 (81.9)	38 (18.1)	1.66 (1.03-2.69)	0.56 (0.27-1.15)

Table 6 (cont)

Characteristics of study patients	Non-severe *P. vivax* malaria subjects *n* (%)	Severe *P. vivax* malaria subjects *n* (%)	cOR (95%CI)	aOR (95% CI)
Hemoglobin (N = 550)				
No anemia (>12 g/dl in females, >13 g/dl in males)	219 (86.2)	35 (13.8)	1	1
Mild anemia (8-12 g/dl in females, 8-13 g/dl in males)	249 (88.6)	32 (11.4)	0.80 (0.48-1.34)	0.62 (0.32-1.20)
Severe anemia (<8 g/dl)	4 (26.7)	11 (73.3)	17.21 (5.19-57.06)	9.30 (1.81-47.77)
Platelet count (N = 550)				
>150,000 cells/μl	80 (92.0)	7 (8.0)	1	1
100,001-150,000 cells/μl	117 (90.0)	13 (10.0)	1.27 (0.49-3.32)	0.85 (0.22-3.25)
≤100,000 cells/μl	275 (82.6)	58 (17.4)	2.41 (1.06-5.49)	2.42 (0.78-7.53)
BUN (N = 527)				
Normal (<20 mg/dl)	436 (86.3)	69 (13.7)	1	1
Mild elevation (20-40 mg/dl)	14 (77.8)	4 (22.2)	1.81 (0.58-5.64)	0.73 (0.13-4.17)
Abnormal (>40 mg/dl)	1 (25.0)	3 (75.0)	18.96 (1.94-184.85)	8.66 (0.28-268.28)

Table 6 (cont)

Characteristics of study patients	Non-severe *P. vivax* malaria subjects *n* (%)	Severe *P. vivax* malaria subjects *n* (%)	cOR (95%CI)	aOR (95% CI)
Bicarbonate (N=518)				
>20 mmol/l	426 (86.8)	65 (13.2)	1	1
≤20 mmol/l	18 (66.7)	9 (33.3)	3.28 (1.41-7.60)	2.80 (0.94-8.32)
Albumin (N=520)				
≥3 mg/dl	424 (87.8)	59 (12.2)	1	1
<3 mg/dl	19 (51.3)	18 (48.7)	6.81 (3.38-13.71)	3.66 (1.19-11.25)
AST (N = 532)				
Normal (0-32 U/l)	325 (90.5)	34 (9.5)	1	1
1-3 times x UNL (33-96 U/l)	121 (74.2)	42 (25.8)	3.32 (2.02-5.46)	2.42 (1.26-4.66)
>3 times x UNL (>96 U/l)	9 (90.0)	1 (10.0)	1.06 (0.13-8.64)	0.75 (0.08-7.61)

aOR: adjusted odds ratio; AST: aspartate aminotransferase; BUN: blood urea nitrogen; CI: confidence interval; cOR: crude odds ratio; g/dl: g/dl: gram per deciliter; G6PD: Glucose-6-phosphate dehydrogenase; mg/dl: milligram per deciliter; mmol/l: millimole per liter; µl: microliter; UNL: upper normal limit; U/l: units/liter; °C: degree Celsius

pregnant women had severe *P. vivax* malaria and the number of subjects (*n*=3) was too small to make any conclusions.

In our study, we found a significant association between fever on admission and severe malaria. *P. vivax* malaria is more likely to cause fever than other malaria species (Dayananda *et al*, 2018). A study from Turkey reported *P. vivax* malaria subjects with high fever had high interleukin-6 and interleukin-10 levels (Zeyrek *et al*, 2006). A study from Indonesia also reported *P. vivax* malaria subjects had high interleukin-6, interleukin-10, E-selectin, plasma angiopoietin-2, and ICAM-1 levels (Yeo *et al*, 2010). *P. vivax* malaria patients with fever may have higher inflammatory cytokine levels which also may be associated with more severe *P. vivax* malaria (Dayananda *et al*, 2018).

In our study, subjects with G6PD deficiency more significantly more likely to have severe *P. vivax* malaria, unlike the results of a study from Pakistan that reported G6PD deficiency was protective against *P. vivax* malaria infection (Leslie *et al*, 2010). A meta-analysis found no association between G6PD deficiency and *P. vivax* malaria infection among Pakistanis, Thais, Burmese and Indonesians (Mbanefo *et al*, 2017). A study of the G6PD-Mahidol (487A) variant, a common variant of G6PD deficiency in Southeast Asia, reported *P. vivax* malaria parasite density was lower among patients with G6PD deficiency, but *P. falciparum* malaria parasite density was not lower among patients with G6PD deficiency (Louicharoen *et al*, 2009). It is unclear why our G6PD deficiency subjects were more likely to have severe malaria in contrast to other studies.

Gametocytes play a role in malaria transmission. In our study, subjects with gametocytemia on admission were more likely to have severe *P. vivax* malaria. Douglas *et al* (2013) reported gametocytemia in patients with *P. vivax* malaria could be found in early malaria infection. McKenzie *et al* (2007) reported gametocytemia was associated with higher initial parasitemia among *P. vivax* malaria patients. It has been reported among patients with *P. vivax* malaria and gametocytemia that they were more likely to have fever, lower hemoglobin and lower albumin levels (Nacher *et al*, 2004), indicating greater severity.

In our study, subjects with hypoalbuminemia were significantly more likely to have severe *P. vivax* malaria. A possible mechanism

leading to severe malaria is acute capillary leakage syndrome, defined as hypoalbuminemia, accompanied by generalized edema, hemoconcentration, hypotension, and the lack of albuminuria (Das *et al*, 2019). Anti-malaria drugs require albumin for transport in the body (Batty *et al*, 2004; Zhu *et al*, 2023). Ninety-three percent of dihydroartemisinin, 88% of artemether and 85-88% of artemisinin are bound to albumin in the serum (Batty *et al*, 2004; Sidhu and Ashton, 1997; Zhu *et al*, 2023). Artesunate, an artemisinin derivative, commonly used for treating severe malaria, including severe *P. vivax* malaria, in intravenous form, is highly protein bound in blood circulation (Veerappan *et al*, 2013). We postulate the pharmacokinetics of these drugs may be affected by hypoalbuminemia resulting in more severe malaria.

In our study, we found a significant association between anemia and severe malaria. It has been estimated that 34 uninfected red blood cells are removed from the circulation by the spleen for every malaria infected red blood cell (Dayananda *et al*, 2018; Douglas *et al*, 2012). This can result in anemia among subjects even with lower parasite densities (Douglas *et al*, 2012; Tjitra *et al*, 2008). Anemia among *P. vivax* malaria patients is associated with higher inflammatory cytokine levels and oxidative stress in the red blood cells leading to extravascular hemolysis (Dayananda *et al*, 2018).

On multivariate analysis we found a significant association between an elevated AST level and severe malaria, similar to other studies from Thailand (Tangpukdee *et al*, 2006; White, 2022). It has been postulated this is due to malarial hepatopathy (Jain *et al*, 2016), defined as a total bilirubin level >3 g/dl and an AST level >3 times the upper normal limit without other causes. Patients with malaria hepatopathy are significantly more likely to die from malaria and more likely to have cerebral malaria, acute respiratory distress syndrome (ARDS), acute kidney injury and shock (Jain *et al*, 2016).

Study limitations

Our study had several limitations. The first was that it was a retrospective review and some of the records were lost or damaged. A second limitation was the limited number of subjects due to fewer malaria cases in Thailand from malaria control efforts. This could have led to not identifying all associations due

to inadequate numbers of subjects to reach statistical significance. A solution for this would be to conduct a multicenter study, increasing the number of subjects to reach statistical significance and allowing the results to be more generalizable. Finally, our study population might be skewed to more severe cases since our institution is a tropical disease referral center.

In summary, 14.2% of our study subjects had severe *P. vivax* malaria. The factors significantly positively associated with severe malaria were: G6PD deficiency, having a fever on admission, having gametocytemia, having severe anemia, having hypoalbuminemia and having an elevated AST level. The factor significantly negative associated with severe malaria was male gender. We conclude, *P. vivax* malaria patients with these factors who do not have severe malaria should be monitored for further deterioration and need urgent treatment. Further studies are need to determine if there are interventions that can prevent non-severe patients with these risk factors from deteriorating to severe infection.

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CONFLICT OF INTEREST DISCLOSURE

The authors declare that we have no conflict of interest.

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