Severe vivax malaria: a prospective exploration at a tertiary healthcare centre in Southwestern India

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ABSTRACT

Plasmodium vivax is recognized to cause severe malaria and mortality. We aimed to determine the proportion of disease severity, the spectrum of complications, underlying non-infectious comorbidities and predictors of severity in monoinfection *P. vivax* malaria among adults at a tertiary healthcare centre in Southwestern India. A prospective cohort study was conducted among microscopically confirmed monoinfection *P. vivax* acute malaria patients aged, ≥ 18 years. Cases with pregnancy and concomitant febrile illnesses including mixed malaria were excluded. Cases were distinguished as either 'severe' or 'non-severe' *P. vivax* malaria as per the definitions laid by the World Health Organization. Of total 511 acute *P. vivax* cases studied, 23.9% (122/511) had severe malaria. The proportion of severity did not vary between microscopy alone and additional nPCR proved monoinfection *P. vivax* subgroups. There was no significant difference ($p = 0.296$) in the occurrence of non-infectious comorbidities among non-severe (9.0%, 35/389) and severe (12.3%, 15/122) vivax groups. Multiple complications despite early parasite clearance resulted in delayed casualty in two cases, indicating overall case fatality rate of 3/1000 cases. Age > 40 years, rising respiratory rate, total bilirubin, serum creatinine and falling hemoglobin were the independent predictors of disease severity in this vivax malaria cohort. Total and direct bilirubin and serum urea had good discriminatory performance for severe vivax malaria. Total bilirubin should be considered as an important prognostic marker while managing *P. vivax* malaria. Patients with multiple complications must be treated cautiously as there may be delayed deterioration leading to mortality despite parasite clearance.

KEYWORDS

Malaria; *Plasmodium vivax*; severe malaria; complicated malaria

Introduction

Plasmodium vivax amounts about half to the global malaria burden outside Africa. South-East Asia staying most endemic added 74% to 13.8 million global vivax malaria burden in the year 2015 [1]. Conversely, a gross underestimation in estimates of World Health Organization (WHO)'s global *P. vivax* burden has been contemplated by peer scientific reviews, which is projected to vary from 70 to 390 million vivax malaria cases a year [2–6]. India with Ethiopia and Pakistan added over 80% to global *P. vivax* burden in the year 2015 [1]. In India, *P. vivax* contributes almost half to the annual burden of malaria [7] and the state of Karnataka records over 70% vivax malaria of its annual malaria burden [8] whereas, in Udupi district, Karnataka, *P. vivax* contributes about 90% to its annual malaria burden [9]. A majority of malaria cases and mortality in India goes unnoticed by formal healthcare facilities due to their inaccessibility, resulting in underestimation of precise burden [10,11]. Moreover, severe malaria poses global public health challenge as more than one-half severe cases don't have access to healthcare facilities [12]. Presuming malaria attributable mortality of 90% at home and 20% in-hospital, the global

annual incidence of severe malaria cases would be about two million [12]. In the year 2015, severe vivax malaria was attributed to cause 3.5–16% of all malaria related mortality outside sub-Saharan Africa [1].

Severe malaria is a medical emergency which warrants aggressive antimalarials and intensive clinical management. In addition to age, pregnancy, parasite species and their susceptibility to a given antimalarial, disease severity is one of the determinants which directs the treatment practices/guidelines [13,14]. The severity of malaria is a function of parasite virulence, the degree of parasitaemia, sequestration and host immune competence which in turn depends on factors viz. age, regional transmission intensity, nutritional status and genetic susceptibility [15–19]. With these variables, the risk and spectrum of malarial complications vary substantially across the globe [12,20,21].

Despite, reasonably convincing evidence [22] of clinical severity caused by inherently pernicious *P. vivax* strains inoculated for treating neurosyphilis patients since early twentieth century, this species, ironically remained tagged with the term 'benign' until recently [1,12]. There have been several accounts [20,23–37] of

severe *P. vivax* malaria worldwide for over last two decades. Previously tagged as benign, *P. vivax* is now globally recognized to cause severe disease and mortality [1,12]. Despite the global recognition, there persist inadequacy in estimates of population attributable risk of vivax malaria related severity and mortality, which necessitates further studies [1,15]. Also, the extent to which underlying comorbidities attribute to severity and mortality in vivax malaria remains uncertain [12]. We aimed to determine the proportion of disease severity, the spectrum of complications, underlying non-infectious comorbidities and predictors of severity in monoinfection *P. vivax* malaria among adults at a tertiary healthcare centre in Southwestern India.

Methods

Study design and patients' selection

A prospective cohort study was conducted among microscopically confirmed monoinfection *P. vivax* acute malaria patients aged, ≥ 18 years, who attended Kasturba Hospital (KH), Manipal from September 2012 till October 2014. Cases with pregnancy and concomitant febrile illnesses including mixed malaria were excluded. Concomitant infectious morbidities were precluded by attending clinicians through differential diagnoses and suggestive routine investigations including blood and urine cultures. This study was integrated to a concurrent therapeutic trial [38] of chloroquine-primaquine (CQ-PQ) combined regimen in monoinfection *P. vivax* acute malaria. The trial [38] included patients microscopically proven to have monoinfection of *P. vivax*, aged, ≥ 18 years, who attended KH, Manipal from September 2012 until October 2014. Of preliminary 161 *P. vivax* cases enrolled, the attending clinicians substituted CQ with artesunate combinations in 36 cases within 48 h prompting their eventual exclusion. None of them had any complication. Finally, a total of 125 participants sustaining the study selection criteria remained in the trial [38]. However, all 161 cases of the trial [38] were included in the current study. While quantitative buffy coat (QBC) test is the mainstay of malaria diagnosis at KH, Manipal, an independent light microscopy examination of Leishman's stained thin and thick blood smears was carried out for participants ($n = 161$) of the concurrent trial [38] to ascertain *P. vivax* monoinfection and to determine the parasite load per μL blood [39].

Ethical consideration

This study was conducted after obtaining an approval (IEC 193/2011) from the institutional ethics committee of Kasturba Medical College and Kasturba Hospital, Manipal University, Manipal. We did not need, a written informed consent for accessing the patients' hospital records (bedside/ambulatory) and a written informed consent was

solely obtained from every participant of the concurrent therapeutic trial [38]. Pertinent data was extracted from patients' hospital records (bedside/ambulatory) during their treatment/hospitalization. Patients' identification and data were anonymized with indirect identification numbers.

Cohort size determination

Previously, exempting 'hyperbilirubinemia' as one of the severity criteria, we reported a 16.9% (95% CI 13.9–19.9%) severity proportion in a retrospective cohort of *P. vivax* malaria [20]. About 14% (81/585) of *P. vivax* cases in the previous study did have hyperbilirubinemia. In this study, we have included 'hyperbilirubinemia' as one of the stand-alone severity criteria as per the revised WHO definition of severe *P. vivax* malaria. Therefore, assuming a prevalence of severity in *P. vivax* malaria to be 31%, with a relative precision of 15% and desired confidence level of 95%, a cohort of 507 *P. vivax* malaria cases was required.

Exposure variables

Cases were distinguished as either 'severe' or 'non-severe' *P. vivax* malaria as per the definition laid by the WHO [13, 40,41] excepting 'prostration'. A case of *P. vivax* malaria was determined as 'severe' if one or more of these occurred: impaired consciousness including coma, seizure/multiple convulsions within 24 hours, respiratory distress (respiratory rate > 32 beats/minute with labored breathing), pulmonary edema or acute respiratory distress syndrome (PE/ARDS; radiological), shock/circulatory collapse (systolic blood pressure < 80 mmHg), acute kidney injury (AKI; 'Injury' as per RIFLE classification and 'renal impairment' as serum creatinine > 3 mg/dL), clinical jaundice with liver dysfunction (more than three times rise in serum transaminases of their upper reference limit i.e. AST > 120 IU/L or ALT > 189 IU/L), abnormal spontaneous bleeding (petechiae, ecchymosis, epistaxis, gum bleed, sub-conjunctival hemorrhage etc.), metabolic acidosis (plasma bicarbonate < 15 mmol/L), severe anemia (haemoglobin < 7 g/dL), hyperbilirubinemia (total bilirubin > 3 mg/dL), hypoglycemia (plasma glucose < 40 mg/dL) and hemoglobinuria (a positive urine dipstick test result without hematuria). The worst value of the said variables recorded during hospitalization was entered for analysis. Other clinical/laboratory parameters and underlying non-infectious comorbidities were assessed as exposure variables for the severity of vivax malaria.

Outcome variables

Diagnosis of a case as 'severe *P. vivax* malaria' was the prime outcome. Besides, mortality and supportive requirements were secondary outcomes.

Confirmation of monoinfection *P. vivax* malaria by nested PCR (nPCR)

An nPCR test [42] was performed to detect *P. vivax* and *Plasmodium falciparum* infections exclusively among those who participated ($n = 161$) in the therapeutic trial [38].

Statistical analyses

Continuous variables were summarized as mean with standard deviation (SD) or median with interquartile range (IQR). Categorical variables were summarized as the frequency with proportion. Non-severe and severe

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Figure 1. Patient's selection and study outcomes.

groups were compared using chi-square or Fisher's exact test and independent *t*-test or Mann Whitney *U* test. Spearman's correlation coefficients (ρ , ρ) were derived to determine correlations of parasite index (parasites/ μ L blood) with complications. Binary logistic regression analysis was performed to determine the odds of factors associated with severity of *P. vivax* malaria. Multicollinearity was checked among variables showing a *p*-value ≤ 0.1 in univariate logistic regression analysis and one of the mutually collinear variables was further included in the multivariate logistic regression model. Variables with a *p*-value < 0.05 in univariate logistic regression analysis were assessed to discriminate a severe vivax case from a non-severe one, by receiver operating characteristic (ROC) curves. Further, whichever variables having good predictive ability (area under ROC > 0.7), of that, an optimum cut-off value was determined with maximum sensitivity and specificity. Poisson regression was performed to determine the association of parasite index with disease severity. Kaplan–Meier survival analysis with log-rank test was performed to determine cumulative probability of survival during hospitalization

for non-severe and severe vivax groups. All tests of significance were two-tailed with a *p*-value less than 0.05 indicating statistical significance. Positive predictive value (PPV) of microscopy for detecting monoinfection of *P. vivax* was determined considering nPCR test as the 'gold standard'. Data analysis was performed using Statistical Package for the Social Sciences version 15.0 (SPSS, South Asia, Bangalore, India).

Results

Figure 1 depicts patients' selection and outcomes of the study. Of total 511 acute *P. vivax* cases studied, 23.9% (122/511) had severe malaria. All severe cases did have complication(s) at their presentation.

Baseline demographic and clinical characteristics

A comparison of relevant demographic and clinical characteristics between non-severe and severe subgroups is summarized in Table 1. Age of the study cohort ranged from 18 to 77 years, severe vivax malaria patients

Table 1. Comparison of demographic and clinical variables between non-severe and severe *P. vivax* malaria from Kasturba Hospital, Manipal, Karnataka, India.

Variables	Non-severe (<i>N</i> = 389) <i>n</i> (%) or mean ($\pm$ SD) or median (IQR)	Severe* (<i>N</i> = 122) <i>n</i> (%) or mean ($\pm$ SD) or median (IQR)	<i>p</i> -value*
Point-of-care			0.005
Ambulatory	40 (10.3)	3 (2.5)	
In-hospital	349 (89.7)	119 (97.5)	
Age in years	32.7 $\pm$ 13.6	39.0 $\pm$ 13.7	<0.001
Gender			1.000
Male	327 (84.1)	103 (84.4)	
Female	62 (15.9)	19 (15.6)	
History			
Fever in days	4 (3, 6)	5 (3, 7)	<0.001
Headache	217 (55.8)	56 (45.9)	0.062
Cough	82 (21.1)	30 (24.6)	0.452
Vomiting	104 (26.7)	48 (39.3)	0.009
Diarrhea	17 (4.4)	6 (4.9)	0.804
Clinical malaria	69 (18.1)	19 (16)	0.680
Comorbidity [^]	35 (9)	15 (12.3)	0.296
Physical examination			
Axillary temperature at presentation ($^{\circ}$ F) [†]	100.4 $\pm$ 1.8	100.3 $\pm$ 1.8	0.402
Pulse rate (beats/minute)	88 $\pm$ 13	90 $\pm$ 14	0.128
Respiratory rate (breaths/minute)	21 $\pm$ 4	24 $\pm$ 10	0.023
Systolic blood pressure (mmHg)	119 $\pm$ 13	115 $\pm$ 17	0.038
Diastolic blood pressure (mmHg)	77 $\pm$ 9	74 $\pm$ 12	0.033
Pallor	42 (10.9)	24 (19.7)	0.020
Icterus	44 (11.4)	68 (55.7)	<0.001
Splenomegaly	116 (30)	46 (37.7)	0.119
Hepatomegaly	67 (17.3)	39 (32)	0.001
Antimalarial treatment			<0.001
CQ-PQ	275 (70.7)	57 (46.7)	
Artesunate combination therapy (ACT)-PQ	114 (29.3)	65 (53.3)	
Empiric antibiotic treatment	27 (6.9)	7 (5.7)	0.835
Length of hospitalization in days	4 (3, 5)	5 (4, 7)	<0.001
Intensive care	2 (0.5)	24 (19.7)	<0.001
Monoinfection <i>P. vivax</i> proven by			1.000
Microscopy alone	277 (71.2)	87 (71.3)	
Microscopy and nPCR test	112 (28.8)	35 (28.7)	

*Categorical variables are summarized as the frequency with proportion whereas continuous variables are summarized as either mean ($\pm$ SD) or median (IQR). Chi-square or Fischer's exact test and Independent sample *t*-test or Mann Whitney *U* test were performed, *p*-value less than 0.05 shows the statistically significant difference and shown in bold font;

[^]As per the guidelines laid by the World Health Organization [13,40,41].

[†]Detailed presentation is made in Table 2.

[†]To convert temperature to $^{\circ}$ C = [$^{\circ}$ F–32] $\times$ 5/9.

were significantly ($p < 0.001$) older in age. The gender ratio was 5.3:1 i.e. 430 male vs. 81 female. However, there was no significant ($p = 1.000$) gender propensity for severe vivax malaria. Severe vivax patients had significantly longer history of fever at presentation ($p < 0.001$), more frequent episodes of vomiting ($p = 0.009$), pallor ($p = 0.020$), icterus ($p < 0.001$) and hepatomegaly ($p = 0.001$). A significant rise in respiratory rate ($p = 0.023$) and fall in systolic ($p = 0.038$) and diastolic blood pressure ($p = 0.033$) was noted among severe vivax patients.

Microscopy vs. nPCR test

The nPCR test revealed 8.1% (13/160) mixed infections (*P. vivax* and *P. falciparum*) in microscopy (both QBC and light microscopy) proven monoinfection *P. vivax* cases. The DNA sample of one participant (0.8%) could not be amplified. However, the proportion of non-severe and severe cases did not vary ($p = 1.000$) in microscopy alone and additional nPCR proved monoinfection *P. vivax*

Table 2. Distribution of non-infectious comorbidities among non-severe and severe *P. vivax* malaria from Kasturba Hospital, Manipal, Karnataka, India.

Comorbidities*	Non-severe (<i>N</i> = 389) <i>n</i> (%)*	Severe [#] (<i>N</i> = 122) <i>n</i> (%)*
Diabetes mellitus	15 (3.9)	7 (5.7)
Hypertension	8 (2.1)	2 (1.6)
Chronic kidney disease	1 (0.3)	1 (0.8)
Liver disease	0	2 (1.6)
Cardiovascular disease	5 (1.3)	0
Pulmonary disease	6 (1.5)	3 (2.5)
Anemia of chronic disease	2 (0.5)	2 (1.6)
Sinusitis	3 (0.8)	0

*Comorbidities were not mutually exclusive and the respective frequencies are mutually inclusive. There was no significant ($p = 0.258$) difference in frequency of individual comorbidities between the groups.; [#]As per the guidelines laid by the World Health Organization [13,40,41].

subgroups. The PPV of microscopy was 91.9% against nPCR for detecting monoinfection *P. vivax* cases.

Patients' treatment

Antimalarials were prescribed only after the confirmation of *P. vivax* parasitaemia by microscopy. Attending clinicians prescribed specific antimalarial regimen by their discretion and the national guideline for the treatment of malaria [14]. CQ-PQ and ACT-PQ were respectively the most frequently ($p < 0.001$) prescribed antimalarials for non-severe (70.7%, 275/389) and severe (53.3%, 65/122) vivax groups (Table 1). There was no significant difference ($p = 0.835$) in the frequency of empiric antibiotic administration between non-severe (6.9%, 27/389) and severe (5.7%, 7/122) vivax malaria (Table 1). The participants of therapeutic trial treated exclusively with CQ-PQ combined regimen did have outstanding (~100%) response [38].

Non-infectious comorbidity

There was no significant difference ($p = 0.296$) in the occurrence of comorbidities among non-severe (9.0%, 35/389) and severe (12.3%, 15/122) vivax groups (Table 1). The occurrence of respective comorbidities in non-severe and severe vivax groups is summarized in Table 2.

Baseline laboratory parameters

A comparison of relevant routine laboratory parameters of non-severe and severe subgroups is summarized in Table 3. Although within normal reference limit i.e. 4000 – 10000 cells/mm³, median total leucocyte count was significantly ($p = 0.026$) higher in severe vivax group. Occurrence of leucopenia did not differ ($p = 0.684$),

Table 3. Comparison of routine laboratory variables between non-severe and severe *P. vivax* malaria from Kasturba Hospital, Manipal, Karnataka, India.

Variables	Non-severe (<i>N</i> = 389) <i>n</i> (%) or mean (±SD) or median (IQR)	Severe [#] (<i>N</i> = 122) <i>n</i> (%) or mean (±SD) or median (IQR)	<i>p</i> -value*
Total leucocyte count (cells/mm ³)	5100 (4100, 6400)	5600 (4200, 7175)	0.026
Leucopenia (<4000 cells/mm ³)	72 (19.4)	20 (17.2)	0.684
Leucocytosis (>10000 cells/mm ³)	7 (1.9)	11 (9.5)	0.001
Total platelet count (cells/mm ³)	89000 (61250, 121000)	50000 (24000, 102000)	<0.001
Thrombocytopenia (<150000 cells/mm ³)	310 (84.2)	104 (91.2)	0.065
Severe thrombocytopenia (<20000 cells/mm ³)	12 (3.3)	18 (15.8)	<0.001
Hemoglobin (g/dL)	13.4 ± 1.9	12.6 ± 2.7	0.002
Hematocrit (%)	40.4 ± 5.7	37.3 ± 8.2	<0.001
Erythrocyte sedimentation rate (mm/1st hour)	19 (11, 36)	32 (12, 63)	<0.001
Random blood sugar (mg/dL)	127 ± 54.3	136.3 ± 55.8	0.117
Total bilirubin (mg/dL)	1.2 (0.8, 1.8)	3.5 (2.4, 5.4)	<0.001
Direct bilirubin (mg/dL)	0.4 (0.3, 0.6)	1.6 (0.7, 3.3)	<0.001
Indirect bilirubin (mg/dL)	0.8 (0.5, 1.1)	1.7 (1.0, 2.6)	<0.001
Serum aspartate aminotransferase (IU/L)	33 (25, 49)	46 (31, 64.7)	<0.001
Serum alanine aminotransferase (IU/L)	32 (21.7, 54)	42 (25.2, 67)	0.004
Serum alkaline phosphatase (U/L)	79 (64, 99.7)	107 (71, 162)	<0.001
Serum urea (mg/dL)	26 (20, 33.5)	36 (28, 52.2)	<0.001
Serum creatinine (mg/dL)	1.1 (0.9, 1.2)	1.2 (1.0, 1.6)	<0.001

*Categorical variables are summarized as the frequency with proportion whereas continuous variables are summarized as either mean (±SD) or median (IQR). Chi-square or Fischer's exact test and Independent sample *t*-test or Mann Whitney *U* test were performed, *p*-value less than 0.05 shows the statistically significant difference and shown in bold font.; [#]As per the guidelines laid by the World Health Organization [13,40,41].

whereas leucocytosis occurred significantly ($p = 0.001$) more frequent in severe vivax group. Thrombocytopenia was noted in both non-severe and severe vivax groups, but it was of a more significant ($p < 0.001$) magnitude in the latter. Also in the severe group, the occurrence of severe thrombocytopenia (<20000 cells/mm³) was significantly ($p < 0.001$) more frequent. There was markedly significant ($p < 0.001$) thrombocytopenia in patients (9.8%, 12/122) with abnormal bleeding [median (IQR); 23000 (18000, 28500)/mm³] as compared to those without bleeding [median (IQR); 84000 (54000, 117500)/mm³]. The severe vivax group had significantly ($p < 0.05$) low mean hemoglobin and hematocrit, whereas erythrocyte sedimentation rate, total and direct bilirubin, serum transaminases and alkaline phosphatase, urea and creatinine were significantly ($p < 0.05$) higher than the non-severe group. Parasite index (parasites/ μ L blood) was determined in 161 participants of therapeutic trial [38,39]. The geometric mean (95% confidence interval, CI) parasite index of non-severe ($n = 120$) and severe ($n = 41$) vivax groups were 1246 (977.3, 1588) / μ L and 1865 (1233, 2821) / μ L respectively. There was no significant association ($p = 0.532$) of parasite index with vivax malaria severity. Also, parasite index did not show a significant correlation with any complication in 41 severe vivax cases of the trial [38].

Study outcomes

Of total 23.9% (122/511) severe vivax malaria as observed in this study, hyperbilirubinemia was the most frequent complication followed by PE/ARDS, AKI, abnormal spontaneous bleeding, clinical jaundice with liver dysfunction, respiratory distress, shock/circulatory

Table 4. Frequency of complications observed among adults (≥ 18 years) with severe P. vivax malaria (N = 122) from Kasturba Hospital, Manipal, Karnataka, India.

Complications*	N (%)
Impaired consciousness including coma	6 (4.9)
Multiple convulsions within 24 h	3 (2.5)
Respiratory distress i.e. respiratory rate >32 beats/minute with labored breathing	10 (8.2)
Pulmonary edema/acute respiratory distress syndrome (radiological)	26 (21.3)
Shock/circulatory collapse i.e. systolic blood pressure <80 mmHg	7 (5.7)
Acute kidney injury	13 (10.7)
Injury (RIFLE-I)	7 (5.7)
Renal impairment (creatinine >3 mg/dL)	6 (4.9)
Clinical jaundice with liver dysfunction i.e. more than three times rise in serum transaminases of their upper reference limit i.e. AST >120 IU/L or ALT >189 IU/L	11 (9.0)
Abnormal spontaneous bleeding (petechiae, ecchymosis, epistaxis, gum bleed, sub-conjunctival hemorrhage etc.)	12 (9.8)
Metabolic acidosis i.e. plasma bicarbonate <15 mmol/L	5 (4.1)
Severe anemia i.e. haemoglobin <7 g/dL	6 (4.9)
Hyperbilirubinemia i.e. total bilirubin >3 mg/dL	80 (65.6)
Hyperbilirubinemia alone [#]	51 (41.8) [#]

*Complications are defined as per the guidelines laid by the World Health Organization [13,40,41]. *Complications were non-mutually exclusive therefore respective frequencies are mutually inclusive.; [#]An exclusive severity determinant.

collapse, severe anemia and impaired consciousness including coma, metabolic acidosis and seizure/multiple convulsions (Table 4). The majority (91.6%, 468/511) of cases required in-hospital care. Duration of hospitalization and frequency of intensive care were significantly ($p < 0.001$) more in severe vivax malaria group (Table 1). Intensive care was provided to 19.7% (24/122), blood product transfusion to 18.8% (23/122), mechanical ventilation to 7.4% (9/122), inotropes to 3.3% (4/122) and hemodialysis to 1.6% (2/122) of severe cases (Figure 1). In two patients who required hemodialysis, serum creatinine was 9.4 and 9.7 mg/dL. Peak serum creatinine values in rest all patients were ≤ 4.0 mg/dL. Notably, over one-third of severity proportion was attributed exclusively to hyperbilirubinemia (Table 4). Majority (69.7%, 85/122) had one complication, followed by two (21.3%), three (5.7%), four (0.8%), five (0.8%) up to six (1.6%) complications. Occurrences of mutually exclusive complications are summarized in Table 5.

Table 5. Occurrence of mutually exclusive complications* and outcome in severe P. vivax malaria (N = 122) from Kasturba Hospital, Manipal, Karnataka, India.

	N (%)	Outcome
<i>One complication [n = 85 (69.7%)]</i>		
Impaired consciousness including coma (ICC)	0	
Seizure/multiple convulsions (SZR)	0	
Respiratory distress (RD)	4 (3.3)	Survived
Pulmonary edema/acute respiratory distress syndrome (PE/ARDS)	12 (9.8)	Survived
Shock/circulatory collapse (SHK)	1 (0.8)	Survived
Acute kidney injury (AKI); all renal impairment	5 (4.1)	Survived
Clinical jaundice with liver dysfunction (CJLD)	4 (3.3)	Survived
Abnormal spontaneous bleeding (AB)	4 (3.3)	Survived
Metabolic acidosis (MA)	1 (0.8)	Survived
Severe anemia (SA)	3 (2.5)	Survived
Hyperbilirubinemia (HBIL)	51 (41.8)	Survived
<i>Two complications [n = 26 (21.3%)]</i>		
RD + PE/ARDS	2 (1.6)	Survived
RD + AKI	1 (0.8)	Survived
AKI + PE/ARDS	1 (0.8)	Survived
AB + ICC	1 (0.8)	Survived
AB + CJLD	1 (0.8)	Survived
MA + ICC	1 (0.8)	Survived
HBIL + RD	1 (0.8)	Survived
HBIL + PE/ARDS	6 (4.9)	Survived
HBIL + SHK	1 (0.8)	Survived
HBIL + AKI	2 (1.6)	Survived
HBIL + CJLD	4 (3.3)	Survived
HBIL + AB	4 (3.3)	Survived
HBIL + SA	1 (0.8)	Survived
<i>Three complications [n = 7 (5.7%)]</i>		
MA + AB + PE/ARDS	1 (0.8)	Survived
HBIL + ICC + SZR	2 (1.6)	Survived
HBIL + SHK + PE/ARDS	1 (0.8)	Survived
HBIL + AKI + SHK	1 (0.8)	Survived
HBIL + CJLD + AKI	1 (0.8)	Survived
HBIL + AB + ICC	1 (0.8)	Survived
<i>Four complications [n = 1 (0.8%)]</i>		
ICC + SZR + SHK + HBIL	1 (0.8)	Survived
<i>Five complications [n = 1 (0.8%)]</i>		
RD + PE/ARDS + CJLD + SA + HBIL	1 (0.8)	Survived
<i>Six complications [n = 2 (1.6%)]</i>		
HBIL + MA + AKI + SHK + RD + PE/ARDS	1 (0.8)	Died
HBIL + SA + MA + AKI + SHK + PE/ARDS	1 (0.8)	Died
Total =	122	

*Complications are defined as per the guidelines laid by the World Health Organization [13,40,41].

In total, two (1.6%) patients succumbed to severe vivax malaria with multiple complications (Tables 5 and 6). Notably, both the casualties did have adequate clinical and parasitological response [43] to the prescribed antimalarials till hospital stay, but their clinical complications progressively worsened culminating in mortality. The survival distribution didn't differ significantly ($p = 0.584$) between non-severe and severe vivax malaria groups (Figure 2).

Table 6. Patients' profile who succumbed to severe *P. vivax* malaria at Kasturba Hospital, Manipal, Karnataka, India.

Variables	Patient 1	Patient 2
Age	66	40
Gender	Male	Female
Monoinfection <i>P. vivax</i> confirmation by nPCR	Yes	Yes
Comorbidity	No	No
Complications*		
HBIL + MA + AKI + SHK + RD + PE/ARDS	Yes	No
HBIL + SA + MA + AKI + SHK + PE/ARDS	No	Yes
Supportive requirements		
Intensive care	Yes	Yes
Blood product transfusion		
Packed red blood cells	No	Yes
Platelets	Yes	Yes
Mechanical ventilation	Yes	Yes
Inotropes	Yes	Yes
Hemodialysis	Yes	No
Time to parasite clearance, days	Day '2'	Day '2'
Defervescence time, hours	0; Afebrile [†]	0; Afebrile [†]
Duration of hospitalization, in days	15	11
Antimalarial medications	CQ, ACT – i.v., PQ	ACT – i.v., PQ

*Complications are defined as per the guidelines laid by the World Health Organization [13, 40, 41]. nPCR, nested PCR; HBIL, hyperbilirubinemia; MA, metabolic acidosis; AKI, acute kidney injury; SHK, shock; RD, respiratory distress; PE/ARDS, pulmonary edema/acute respiratory distress syndrome; SA, severe anemia. i.v., intravenous;

[†]Afebrile at admission and remained so thereafter.

Predictors of disease severity in *P. vivax* malaria

Age above 40 years, rising respiratory rate, total bilirubin, serum creatinine and falling hemoglobin were the independent predictors of disease severity in this vivax malaria cohort with statistically significant odds (Table 7).

Variables with significant discriminatory efficacy for severe vivax malaria

Of all variables having a p -value < 0.05 in univariate logistic regression analysis (Table 7), only total and direct bilirubin and serum urea occupied above 70% area under the curve (AUC) in a ROC analysis for severe vivax (Figure 3). AUC occupancy of rest all variables was less than 70%; hence excluded, Table 8. In ROC analysis, even after omitting severe vivax cases with 'hyperbilirubinemia' as the sole complication, total and direct bilirubin and serum urea did sustain above 70% AUC occupancy.

The maximum sensitivity, 80.2% and sepecificity, 78.3% of a total bilirubin i.e. 1.85 mg/dL were noted to discriminate a severe vivax case in this study cohort. Whereas, after excluding cases with hyperbilirubinemia, the maximum sensitivity and specificity were 72.3 and 61.1% respectively for a total bilirubin as 1.65 mg/dL to discriminate a severe vivax case. Besides, total bilirubin as 2.55 mg/dL had 74.0% sensitivity and 94.8% specificity for discriminating severe vivax malaria in this study cohort. In vivax cohort without 'hyperbilirubinemia', total bilirubin as 2.55 mg/dL had 53.8% sensitivity and 82.3% specificity. ereAlso, the maximum sensitivity, and specificity of 68.3 and 68.5% were noted for serum urea, 30.5 mg/dL to discriminate as severe vivax cases.

Figure 2. Kaplan–Meier plot showing cumulative survival probability during hospitalization for non-severe and severe vivax groups.

Table 7. Predictors of disease severity in *P. vivax* malaria at Kasturba Hospital, Manipal, Karnataka, India.

Variables	Odds ratio [95% confidence interval] [†]	<i>p</i> -value [†]	Adjusted odds ratio [95% confidence interval] [†]	<i>p</i> -value [†]
Age				
Up to 40 years	Reference		Reference	
Above 40 years	2.12 [1.39–3.23]	<0.001	2.38 [1.06–5.35]	0.036
More than 3 days history of fever	1.70 [1.08–2.69]	0.023	1.26 [0.54–2.94]	0.589
Vomiting	1.78 [1.16–2.72]	0.008	1.44 [0.63–3.27]	0.384
Pallor	2.01 [1.16–3.47]	0.013	¶	
Icterus	9.79 [6.08–15.75]	<0.001	¶	
Hepatomegaly	2.25 [1.42–3.58]	0.001	0.67 [0.25–1.76]	0.412
Respiratory rate (breaths/minute)	1.08 [1.03–1.13]	0.002	1.15 [1.05–1.26]	0.002
Systolic blood pressure (mmHg)	0.98 [0.97–0.99]	0.015	0.98 [0.95–1.01]	0.164
Diastolic blood pressure (mmHg)	0.97 [0.95–0.99]	0.012	¶	
Total leucocyte count/500 (cells/mm ³)	1.06 [1.01–1.10]	0.009	¶	
Leucocytosis (>10000 cells/mm ³)	5.45 [2.06–14.40]	0.001	5.20 [0.93–29.22]	0.061
Total platelet count/10000 (cells/mm ³)	0.89 [0.84–0.93]	<0.001	¶	
Severe thrombocytopenia (<20000 cells/mm ³)	5.56 [2.59–11.95]	<0.001	0.86 [0.14–5.17]	0.871
Hemoglobin (g/dL)	0.84 [0.76–0.92]	<0.001	0.80 [0.67–0.95]	0.013
Hematocrit (%)	0.93 [0.90–0.96]	<0.001	¶	
Erythrocyte sedimentation rate (mm/1st hour)	1.02 [1.01–1.03]	<0.001	¶	
Total bilirubin (mg/dL)	5.55 [3.91–7.86]	<0.001	6.93 [4.32–11.11]	<0.001
Direct bilirubin (mg/dL)	11.83 [6.50–21.56]	<0.001	¶	
Hyperbilirubinemia [#]	5.27 [3.02–9.18]	<0.001	¶	
Serum alanine aminotransferase (IU/L)	1.01 [1.00–1.01]	0.057	1.01 [0.99–1.01]	0.115
Serum alkaline phosphatase (U/L)	1.02 [1.01–1.02]	<0.001	¶	
Serum urea (mg/dL)	1.06 [1.04–1.07]	<0.001	¶	
Serum creatinine (mg/dL)	8.50 [4.25–17.00]	<0.001	5.20 [1.85–14.64]	0.002

[†]Univariate and multivariate logistic regression analysis was carried out, significant *p*-value i.e. <0.05 are shown in bold face and odds ratios which did not overlap the null value of confidence interval i.e. 1 are shown in bold face. Only those variables are shown which yielded *p* ≤ 0.1 in univariate regression analysis.

[#]Cases with hyperbilirubinemia 'alone' were excluded.

[¶]Variables were not included in the multivariate logistic regression model due to multicollinearity: systolic blood pressure = diastolic blood pressure; total leucocyte count/500 = leucocytosis; hemoglobin = hematocrit, pallor, erythrocyte sedimentation rate; total bilirubin = icterus, direct bilirubin, hyperbilirubinemia, alkaline phosphatase, and urea; serum urea = serum creatinine.

Figure 3. ROC curve depicting discriminatory efficacy of total bilirubin (AUC, 95% CI = 0.900, 0.853–0.946), direct bilirubin (AUC, 95% CI = 0.855, 0.798–0.912) and serum urea (AUC, 95% CI = 0.736, 0.674–0.797) for severe vivax malaria.

Discussion

While *P. vivax* is recognized globally to cause severe malaria and mortality, there remains paucity of information in estimates of population attributable risk of severity and mortality. Hospital based studies with

proven parasitaemia deliver most reliable estimates on the incidence of severe malaria and mortality in a given population [12]. The majority (82.2%, 423/511) of this study cohort is likely non-immune as they did never have clinical malaria in the past but all were symptomatic with fever or history of fever.

Table 8. Receiver operating curve characteristics of variables for severe vivax malaria.

Test variables	Area	95% confidence interval	Std. error ^a	p-value ^b
Respiratory rate (breaths/minute)	0.598	0.523–0.673	0.038	0.007
Systolic blood pressure (mmHg)	0.550	0.483–0.618	0.035	0.110
Diastolic blood pressure (mmHg)	0.556	0.490–0.622	0.034	0.076
Hemoglobin (g/dL)	0.592	0.528–0.655	0.032	0.004
Hematocrit (%)	0.615	0.551–0.678	0.032	<0.001
Total platelet count/10000 (cells/mm ³)	0.690	0.628–0.751	0.032	<0.001
Total leucocyte count/500 (cells/mm ³)	0.553	0.480–0.626	0.037	0.142
Erythrocyte sedimentation rate (mm/1st hour)	0.620	0.548–0.692	0.037	0.001
Total bilirubin (mg/dL)	0.900	0.853–0.946	0.024	<0.001
Direct bilirubin (mg/dL)	0.855	0.798–0.912	0.029	<0.001
Serum alkaline phosphatase (U/L)	0.650	0.576–0.724	0.038	<0.001
Serum Urea (mg/dL)	0.736	0.674–0.797	0.031	<0.001
Serum creatinine (mg/dL)	0.664	0.591–0.737	0.037	<0.001

^aStandard error under the nonparametric assumption.^bNull hypothesis: true area = 0.5.

Baseline characteristics

Higher propensity to contract malaria infection in men has been a consistent observation from this study region [9,20,34,35,38,39]. Male preponderance in malaria population has also been reported from other places in India [44–46] and elsewhere [47,48]. In contrast, female predominance was found in vivax malaria in Papua, Indonesia [36]. But, there is no precise elucidation for gender propensity in respective malaria population. Nonetheless, volatile skin emanations [49–51], HLA genotype (Cw*07) [49], skin microbiota composition [52,53] and alcohol consumption [54] have been reported to modulate attractiveness and biting preferences of mosquito vectors to human. Also, steroid hormone dehydroepiandrosterone sulfate (DHEAS) is linked to protection from *P. falciparum* in post-pubertal individuals [55,56].

Significant differences ($p < 0.05$) in baseline clinical and laboratory parameters as summarized in Tables 1 and 3 do implicate underlying perniciousness and decompensation in severe vivax group. Significantly longer duration of fever at the presentation in severe vivax suggests delayed diagnosis/treatment resulting development of complications. A substantial proportion of non-severe cases required hospitalization and intensive care. These cases were admitted in view of mild-moderate anemia, thrombocytopenia, hyperemesis, hypotension, prostration, clinical jaundice and uncontrolled non-infectious comorbidities viz. diabetes mellitus, hypertension etc. In-hospital admission and intensive care, in particular, are the consequences of malarial severity, however, in routine clinical practice, these consequences have been

reported [20,35,37] in otherwise non-severe malaria cases. In a hospital based study [37] from Piura, Peru, of 6502 vivax patients 106 were hospitalized, of whom 25 (23.6%) were non-severe cases. It is not clear that to what extent an affluent availability and affordability of health-care facilities to a given population affect the estimate of in-hospital admission in otherwise non-severe malaria. Yet, the impact of prompt in-hospital care on superior recovery and minimal mortality can't be negated [12,35]. In this regard, malarial mortality at our study centre have been consistently minimal [20,35] and is comparable with some of the best worldwide [37,57–59].

Severity proportion and spectrum

An estimate of severity depends greatly on the definitions of severity used in a study. Previously, exempting hyperbilirubinemia as one of the determinants, we reported [20] 16.9% [95% CI 13.9–19.9%] severity in a cohort of 585 microscopically proven monoinfection *P. vivax* acute malaria cases from KH, Manipal. In the present study, severity proportion appears fairly higher than the previous study [20]. However, after excluding severe cases defined solely by hyperbilirubinemia from the present study (Table 4), the severity proportion (13.9% [95% CI 10.9–16.9%]) appears quite similar to the previous study [20]. This implies to the almost steady occurrence of severity in *P. vivax* infected adult population catered by KH, Manipal during the earlier study [20] periods i.e. January 2007–December 2011 and present study period i.e. September 2012–October 2014. In a multicenter study, Siqueira et al. [60] have reported the severity of 33.9% (157/462) in Bikaner, India and 12.6% (40/316) in Manaus, Brazil among PCR proven vivax malaria population by excluding hyperbilirubinemia as an isolated severity criterion. Among other series with hyperbilirubinemia as one of the severity determinants, Limaye et al. [61] from Mumbai, India reported 14.8% (50/338), Sarkar et al. [62] from Kolkata, India reported 22.2% (200/900), Arévalo-Herrera et al. [63] from Colombia reported 6.8% (46/673) and Zubairi et al. [59] from Karachi, Pakistan reported 37.5% (111/296) severity in vivax malaria.

The severity spectrum as observed in this study ranged across standard malarial complications [13,40,41] except, hemoglobinuria and hypoglycemia. In our previous study [20], hemoglobinuria did occur in 6.1% (6/99) of severe vivax cohort. Hemoglobinuria in severe vivax malaria is a rare occurrence usually associated with glucose-6-phosphate dehydrogenase deficiency and oxidative stress by antimalarials [12]. Although less frequent, hypoglycemia (<40 mg/dL) in severe vivax malaria in adults has been reported from different places e.g. India [64], Pakistan [59] and Sudan [57]. Notably, exclusive combinations of complications (up to six) observed in this vivax cohort is higher than our previous study [20] cohort (up to four). In other studies, severe vivax syndrome included up to seven complications by Limaye

et al. [61], six complications by Sarkar et al. [62], four complications by Nadkar et al. [46], eleven complications by Arévalo-Herrera et al. [63] and ten complications by Zubairi et al. [59]. Apart from distinct occurrences of observed complications, the disparity in severe vivax syndrome across studies is also due to adaptations in definitions of severity.

Predictors of severity

As shown in Table 7, except serum alanine aminotransferase rest all variables had significant odds of severity in univariate logistic regression analysis. However, multivariate logistic regression analysis revealed 'age >40 years', rising respiratory rate, total bilirubin, serum creatinine and falling hemoglobin as the independent predictors of severity in this study cohort. Whereas rising respiratory rate, falling systolic blood pressure, leucocytosis and hematuria were found to have an independent association with vivax malaria severity in our previous study [20]. Increasing age was associated with vivax severity in our previous study [20] and other studies [37,55] as well. Usually, younger children are found to be vulnerable to severe vivax malaria. However, in this adult population which is likely non-immune, vulnerability for severe malaria could be partly explained by the fact that majority (60%, 30/50; $p < 0.001$) of participants with non-infectious comorbidities were aged above 40 years. Also, although statistically insignificant ($p = 0.110$), median (IQR) parasite count in patients above 40 years i.e. 1850 (779, 4810)/ μL was much higher than patients up to 40 years i.e. 1220 (505, 3184)/ μL , suggesting higher inflammatory response induction resulting severity. While usually seen in children and pregnancy, it is interesting to note falling hemoglobin as an independent predictor of severity in this adult vivax malaria cohort. Falling hemoglobin was found to be associated with severe vivax by univariate logistic regression analysis in our previous study [20]. All hemoglobin values were recorded at presentation or the worst values during hospitalization exempting post transfusion hemoglobin values. None of the females were pregnant as it was an exclusion criterion in this study. Also, except two cases of anemia of chronic diseases (Table 2), none of the anemic patients with severe malaria had underlying hemoglobinopathy or marked hemolysis or infectious comorbidity. Serum creatinine is known to have the best prognostic efficacy for renal replacement therapy and outcomes [12]. In our previous study [34], vivax malaria patients with mild AKI (serum creatinine >1.6 mg/dL) had significantly higher odds of severe anemia and blood transfusion, also, occurrence of jaundice, PE/ARDS, requirement of mechanical ventilation, intensive care and prolonged hospitalization were significantly more frequent in vivax patients with mild AKI. Malarial hyperbilirubinemia is known to be a manifestation of hemolysis and liver dysfunction [12]. Since haemolysis is not usually as severe as to cause

significant hyperbilirubinemia, most of these patients actually have some hepatocyte necrosis as evidenced by the mild to moderate increase in liver transaminases with subsequent cholestasis [65]. Also, it has been envisaged that hyperbilirubinemia could be associated with acalculous cholecystitis especially in patients with vomiting and complaint of upper abdominal pain [66]. In this study, total, direct and indirect bilirubin values were significantly elevated in severe vivax malaria than non-severe malaria, Table 3. But, in non-severe vivax malaria, unconjugated bilirubinemia was significantly ($p < 0.001$) more predominant whereas, in severe vivax malaria, there was no significant ($p = 0.057$) difference in median (IQR) indirect and direct bilirubin, Table 3. Of note, conjugated bilirubinemia was significantly ($p = 0.014$) more profound in severe vivax cases with 'hyperbilirubinemia', implicating simultaneous liver dysfunction. Total bilirubin being an independent predictor of severity in this vivax malaria cohort, it had good discriminatory performance for severe malaria. Total bilirubin as 2.55 mg/dL had 74.0% sensitivity and 94.8% specificity for discriminating a severe vivax malaria, which is better than 47.0% sensitivity as reported by Siqueira et al. [60] for 2.50 mg/dL total bilirubin. Clinicians may note that in routine practice, total bilirubin must be considered as one of the prognostic indicators in vivax malaria.

Although unspecific, mild to severe thrombocytopenia is a hallmark of all forms of malaria, which is usually self-limiting following parasitaemia clearance. In the present study, there was significant ($p < 0.001$) thrombocytopenia in patients with abnormal bleeding as compared to those without bleeding. In malaria, though thrombocytopenic bleeding diathesis is uncommon [67,68], clinicians often consider and manage such cases as severe malaria because of their higher propensity to evolve into severe form [69].

Discordant antimalarial prescription practice

Discordant usage of chloroquine and artemisinin derivatives (monotherapy or in-combinations) in all forms of malaria in children is reported by Singh et al. [70] from Moradabad, Northern India. In this study from Southern India, a discordant antimalarial prescription in routine clinical practice is being evident (Table 1). While ACT-PQ prescription to non-severe vivax malaria corresponds with inclusive antimalarial treatment guidelines [13,41], it does seem a consequence of underlying perniciousness viz. mild-moderate anemia, thrombocytopenia, hypotension, clinical jaundice, non-infectious comorbidities and an overt notion of probable emergence or importation of CQ resistant *P. vivax* [38,71,72]. Besides, clinicians' sensitization to the therapeutic trial [38] might have been one of the factors resulting discordant CQ-PQ prescriptions in some severe vivax patients. Whatsoever seemed the explanations of this discordance, antimalarials' must be prescribed prudently as per the standard guidelines.

Strengths and limitations

P. vivax culpability to be the sole cause of disease severity in this cohort is very high as all major febrile illnesses were ruled out by routine screening and those with non-infectious comorbidities did not show a propensity to develop severe malaria. Although we could not confirm monoinfection *P. vivax* by PCR test in the whole study population, nPCR confirmation of *P. vivax* monoinfection in 161 cases significantly strengthens the liability of *P. vivax* as the sole cause of severity. As microscopy had good PPV of 91.9% against nPCR for detecting monoinfection *P. vivax*, in rest of the cases also, the likelihood of *P. vivax* to be the sole cause of severity remains substantially high. Estimates of this study are derived using robust statistical analyses. Of note, the severity definitions adopted in this study are essentially for *P. falciparum* malaria but the same has been advocated to be adopted for other malaria parasites as well, disregarding hyperparasitaemia for *P. vivax* malaria. Besides, WHO definition [41] of severe malaria did show good sensitivity in predicting severe *P. vivax* malaria among children in Brazilian Amazon [73]. Among apparent limitations, natural course/temporal pattern of severity development could not be studied owing to the presence of complication(s) in all severe cases at their presentation. This might be a reflection of the study setting being a tertiary care referral centre. The nPCR test was conducted to rule out only *P. vivax* and *P. falciparum* infections considering our study region endemicity exclusive to these species. Also, in cases with bleeding diathesis, investigations to rule out underlying coagulopathy were not performed.

Conclusions

P. vivax infection causes severe malaria in substantial proportion among adults. Age above 40 years, rising respiratory rate, total bilirubin, serum creatinine and falling hemoglobin were the independent predictors of disease severity. Patients with multiple complications must be treated cautiously as there may be delayed deterioration leading to mortality despite parasite clearance. Total bilirubin should be considered as an important prognostic marker while managing *P. vivax* cases.

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