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A prospective study from south India to compare the severity of malaria caused by *Plasmodium vivax*, *P. falciparum* and dual infection

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ABSTRACT

Background & objectives: Traditionally, *Plasmodium falciparum* has been attributed to cause severe malaria, whereas *P. vivax* is considered to cause "benign" tertian malaria. Recently, there has been an increasing body of evidence challenging this conviction. However, the spectrum and degree of severity of the disease caused by *P. vivax*, as per World Health Organization (2012) remains unclear. Thus, in this prospective study, we aimed at comparing the severity of malaria caused by *P. vivax*, *P. falciparum* and dual infection.

Methods: Adult patients presenting to Christian Medical College, Vellore from October 2012 to September 2013 with microscopically confirmed malaria were included in the study. Their clinical and laboratory parameters were recorded and analyzed. Paired *t*-test and chi-square with 95% CI and post-hoc analyses using the Scheffé post-hoc criterion were used to assess the statistical significance at the level of α <0.05.

Results: In total, 131 cases of malaria were identified during the study period, comprising 83 cases of *P. vivax*, 35 cases of *P. falciparum* and 13 cases of mixed vivax and falciparum infections. The spectrum and degree of hematological, hepatic, renal, metabolic, central nervous system complications of vivax malaria was not different from that of falciparum group. Thrombocytopenia and hyperbilirubinemia were the most common laboratory abnormalities identified in all the groups.

Interpretation & conclusion: This cross-sectional comparative study clearly demonstrates that clinical features, complications and case-fatality rates in vivax malaria can be as severe as in falciparum malaria. Hence, vivax malaria could not be considered benign; and appropriate preventive strategies along with antimalarial therapies should be adopted for control and elimination of this disease.

Key words Mixed infection; India; *Plasmodium vivax*; severe malaria

INTRODUCTION

According to the World Malaria Report 2014, India accounted for nearly 58% of malaria cases in the Southeast Asia Region[1]. Out of 0.88 million cases, 53% cases were due to *Plasmodium falciparum* and 47% were due to *P. vivax*. It has been long held that *P. falciparum* is responsible for severe malaria and *P. vivax* infection is deemed to run a benign course. Over last decade there have been growing evidence that *P. vivax* monoinfection itself can cause multiorgan dysfunction and increased mortality than otherwise held earlier[1-6]. Gradual emergence of chloroquine resistant malaria in Southeast Asia has made it one of the largest parasitic diseases of public health concern. The Urban Malaria Scheme (UMS), launched in 1971, identifies Vellore as a high risk town owing to its industrial setup and water scarcity[1]. In a study on 1416 patients with acute undifferentiated febrile illness from various secondary hospitals of India in 2014, the prevalence of *P. falciparum*, *P. vivax* single infection and dual infection rates were 46, 38 and 11% respectively[7].

Hence, a hospital-based prospective observational study was conducted to describe the clinical profile, laboratory abnormalities, organ-wise complications and outcome of vivax malaria. The severity of the illness with that of falciparum and mixed malaria was also compared.

MATERIAL & METHODS

A prospective study was conducted at Christian Medical College Hospital, Vellore; which is a 2700 bedded tertiary care center in Tamil Nadu. The hospital receives patients from Vellore and neighbouring districts in Tamil Nadu and Andhra Pradesh. Adult patients (age > 16 yr) presenting with an acute febrile illness to the emergency department or general medicine outpatient department from 1 October 2012 through 30 September 2013 were included in the study. The study proposal was cleared by the Institutional Review Board (IRB) and Ethics Committee (EC) of the institution. A written informed consent was obtained from all patients willing to participate in the study. In case

of an obtunded patient, the consent was obtained from the nearest relative. All included patients were investigated to establish an etiological diagnosis. The demographic details, clinical features, relevant hematological and biochemical investigations were recorded in a pre-designed data abstraction sheet. The sequential organ failure assessment (SOFA) score was calculated for all patients at the time of presentation based on the involvement of the six system variables (respiratory, cardiovascular, hepatic, coagulation, renal and neurological systems)[8]. Parasitological diagnosis of malaria was established by microscopic examination of the Giemsa stained peripheral blood thick and thin smears. Other infectious etiologies for acute febrile illness, like dengue (IgM-IgG ELISA; Dengue Duo Cassette, PanBio Ltd), enteric fever (Typhidot IgM and IgG; Malaysia Bio-Diagnostics Research Sdn, Malaysia), scrub typhus (IgM ELISA; PanBio Ltd, Brisbane, Australia) and leptospirosis (IgM ELISA; Virion Serion GmbH, Germany) were ruled out.

Statistical analysis was performed using SPSS software version 16.0. Descriptive data are given as mean (SD) or as median (range). Chi-square test or Fisher's exact test was used to compare dichotomous variables and *t*-test or Mann–Whitney test was used for continuous variables as appropriate. The differences between the groups were analyzed by univariate analysis and their 95% confidence intervals were calculated. For all tests, including post-hoc analyses using the Scheffé post-hoc criterion for significance, *p*-value of 0.05 or less was considered statistically significant.

RESULTS

Demographic and seasonality of malaria cases

During the one year study period, 131 patients were diagnosed to have malaria based on the microscopic examination of the peripheral blood film. *P. vivax, P.* falciparum and mixed infection cases with both the species were 83 (63%), 35 (27%) and 13 (10%) respectively. A total of 87 (66%) cases were detected during the rainy months of July–November and the remaining cases occurred sporadically throughout the year. All 92 (70.2%) patients were the residents of the state of Tamil Nadu, while 33 (25.2%) patients were from Andhra Pradesh, and 6 (4.6%) patients from other states. About 36% of the patients were manual labourers and 13% were agricultural workers. There were four pregnant women in the vivax group and one in the falciparum group.

Clinical features and complications

The mean age (±SD) of the patients were 32.4 ± 12.7 35.9 ± 13.2 and 34.1 ± 11 yr in the vivax, falciparum and mixed infection group respectively, and 66 (79.5%), 22 (62.9%) and 10 (76.9%) were below the age of 40 yr (p = 0.16). There was significant male preponderance observed in each group. The median duration of fever was 6.6 ± 3 days in the *P. vivax* group and 6.1 ± 2.8 days in the *P. falciparum* group which was not statistically different. The demographic features and organ involvement are shown in Table 1.

Table 1. Comparative clinical features and complications of malaria across the three groups of *Plasmodium* species

Clinical features and organ involvement	*P. vivax*	*P. falciparum*	Mixed infection
Age (mean years ± SD)	32.4 ± 12.7	35.9 ± 13.2	34.1 ± 11
Gender Male	74 (89.2)	26 (74.3)	11 (84.6)
Female	9 (10.8)	9 (25.7)	2 (15.4)
Duration of fever (mean days ± SD)	6.6 ± 3	6.1 ± 2.8	8.5 ± 4.3
Severe thrombocytopenia (Platelets <20,000/mm^3)	10 (12.3)	7 (20)	2 (15.4)
Abnormal bleeding	2 (2.4)	4 (11.4)	0
Severe anemia (Hemoglobin < 5 g/dl*)	0	2 (5.7)	0
Jaundice (Total bilirubin ≥ 3 mg/dl**)	16 (20)	13 (37.1)	6 (46.2)
Renal failure (Creatinine ≥ 3 mg/dl**)	1 (1.2)	2 (5.7)	0
Acidosis (Bicarbonate < 15 mEq/dl***)	4 (6.4)	6 (19.4)	0
CNS involvement†	5 (6)	3 (8.6)	1 (7.7)
ARDS‡	2 (2.4)	3 (8.6)	0
Circulatory collapse (SBP ≤ 70 mm Hg)	1 (1.2)	2(5.7)	0
Death	1 (1.2)	1 (2.9)	0

*Gram per deciliter; **Milligrams per deciliter; ***Milliequivalents per deciliter; †Central nervous system involvement as evidenced by the presence of seizures and/or alteration of the sensorium; ‡ARDS = Adult respiratory distress syndrome; Figures in parentheses indicate percentages.

Hematological complications

As per the WHO report on management of severe malaria (2012), platelet count ≤ 20,000 cells/mm^3 and/or hemoglobin < 5 g/dl is described as severe malaria. In our study, the observed mean platelet count (± SD) was lowest in the mixed infection group (52,462 ± 32,235 cells/mm^3) followed by falciparum and vivax groups and these differences were not statistically significant. Severe thrombocytopenia was present in 10 (12.3%), 7 (20%) and 2 (15.4%) patients with *P. vivax, P. falciparum* and mixed infections, respectively which was not statistically significant indicating that the vivax malaria (monoinfection or co-infection with falciparum) can cause similar degree and proportion of thrombocytopenia to that of falciparum monoinfection. Despite the observed thrombocytopenia, overt bleeding manifestations occurred only in 2 (2.4%) patients in the vivax group as compared to 4 (11.4%) patients in the falciparum group. In comparison to the mean hemoglobin of 13.1 ± 4.1 g/dl in the *vivax* group, the hemoglobin was significantly lower in the falciparum group (11.1 ± 2.5 g/ dl, $p = 0.029$) and showed a trend towards significance in the mixed infection group (10.6 ± 2.6 g/dl, $p = 0.068$). None of the patients with vivax infection had hemoglobin < 5 g/dl while 2 (5.7%) patients with falciparum had Hb < 5 g/dl. The white blood cell counts were not statistically different across the three groups of malaria (Table 2). These results clearly show that vivax malaria can cause similar degree of hematological complications as compared to falciparum malaria.

Hepatic dysfunction

The mean total bilirubin was 2.6 ± 3.8, 4.2 ± 4.5 and 2.8 ± 1.4 mg/dl and clinical jaundice (total bilirubin ≥3 mg/dl) was noted in 16 (20%), 13 (37.1%) and 6 (46.2%) patients with *P. vivax, P. falciparum,* and mixed infections, respectively. When compared among the groups, no statistical difference was found. Total serum protein level was significantly lower in the falciparum group as compared to vivax group (6.4 ± 1 *vs* 6.9 ± 0.9 g/dl, $p = 0.006$). As shown in Table 2, aspartate transaminase was

Table 2. Comparison of the laboratory investigations among the three groups of *Plasmodium* species

Laboratory parameter	*P. vivax*	*P. falciparum*	Mixed infection	*P*-value between groups		
				Vivax–Falciparum	Falciparum–Mixed	Vivax–Mixed
Hemoglobin (g/dl)	13.1 ± 4.1	11.1 ± 2.5	10.6 ± 2.6	0.03	0.89	0.07
Total WBC count (cells/mm^3)	6220 ± 2984	5891 ± 2658	5754 ± 2683	0.85	0.99	0.86
Platelet count (cells/mm^3)*	63,000	37,000	58,000	0.09	0.99	0.28
	(41,500 – 104,500)	(21,000 – 79,000)	(26,000 – 72,500)			
Creatinine (mg/dl)	1.2 ± 0.4	1.5 ± 1	1.2 ± 0.4	0.07	0.29	0.99
Urea (mg/dl)*	33	40	32.5	0.03	0.29	0.99
	(26 – 47.8)	(26 – 89)	(28.3 – 50)			
Sodium (mEq/l)	134.6 ± 4.9	133.2 ± 4.7	131.6 ± 4.4	0.38	0.62	0.38
Potassium (mEq/l)	3.9 ± 0.5	3.9 ± 0.6	4.1 ± 0.6	0.77	0.8	0.48
Bicarbonate (mEq/l)	21.5 ± 4.1	18.9 ± 5.2	21.6 ± 4.2	0.04	0.22	0.99
Total bilirubin (mg/dl)*	1.55	2.5	2.9	0.13	0.54	0.98
	(1.10 – 2.45)	(1.30 – 5.5)	(1.55 – 3.9)			
Direct bilirubin (mg/dl)*	0.67	0.80	0.8	0.07	0.32	0.99
	(0.40 – 1.1)	(0.40–3.6)	(0.45 – 2.3)			
Total protein (g/dl)	6.9 ± 0.9	6.4 ± 1	6.4 ± 0.7	0.006	1	0.09
Albumin (g/dl)	4.4 ± 4.9	3.2 ± 0.6	3.2 ± 0.6	0.35	1	0.62
AST/SGOT (IU/L)**	27	45	48	<0.001	0.55	0.07
	(19.3 – 40)	(28 – 94)	(29.5 – 81.5)			
ALT/SGPT (IU/L)***	17	31	38	0.29	0.02	<0.001
	(11 – 33)	(19 – 46)	(17 – 81.5)			
Alkaline phosphatase (IU/L)	82.6 ± 35.3	93.9 ± 38	83.2 ± 33.7	0.30	0.66	0.99

*Median (Inter-quartile range); **AST/SGOT = Aspartate transaminase/Serum glutamic oxaloacetic transaminase; *** ALT/SGPT = Alanine transaminase/Serum glutamic pyruvic transaminase.

significantly higher in the falciparum group when compared to the vivax group (73.1 ± 68.3 *vs* 31.8 ± 15.7 IU/L; $p<0.001$) while alanine transaminase was significantly higher in the mixed infection group as compared to either vivax or falciparum group (68 ± 92.5 *vs* 25.1 ± 12.7 IU/L and 36.3 ± 21.9 IU/L; $p<0.001$ and 0.022 respectively. The mean serum alkaline phosphatase was similar in all the three groups. Thus, it was concluded that the level of hyperbilirubinemia and proportion of clinical jaundice, which are the markers of severity of malaria, were similar in vivax and falciparum infection.

Renal failure and acidosis

The mean serum creatinine level was similar across the three groups. Although, WHO doesn't recommend any cut-off for creatinine level; at the arbitrary cut-off of 3 mg/dl, the proportion of patients having creatinine level >3 mg/dl was 1 (1.2%) in the vivax group as compared to 2 (5.7%) in falciparum group, which was not statistically different. However, the mean level of serum urea was 68.7 ± 73.7 mg/dl in the falciparum group, which was significantly higher than that of vivax group (40.2 ± 25.2 mg/dl, $p=0.026$) but not from the mixed infection group (42.4 ± 26.5, $p=0.294$). Similarly, the mean venous bicarbonate level was significantly lower among the falciparum infected group as compared to the vivax infection group (18.9 ± 5.2 *vs* 21.5 ± 4.1 mEq/dl, $p=0.039$) but not statistically significant as compared to the mixed infection group (21.6 ± 4.2 mEq/dl, $p=0.224$). At an arbitrary cut-off of venous bicarbonate <15 mEq/dl, the proportion of patients in falciparum group was higher (6, 19.4%) than vivax group (4, 6.4%) which showed a tendency towards significant ($p=0.06$).

Central nervous system (CNS) and cardiopulmonary complications

The incidence of alteration in mental status and/or seizure was 5 (6%), 3 (8.6%) and 1 (7.7%) in the vivax, falciparum and mixed infection group respectively, which was not statistically different. Similarly, the proportion of patients with adult respiratory distress syndrome and hypotension (SBP ≤ 70 mm Hg) in vivax and falciparum group was not statistically different. These results indicate that *P. vivax* can cause severe malaria similar to the *P. falciparum* with respect to CNS and cardiopulmonary complications.

Outcome

In the vivax group, 58 (70%) patients were managed on an outpatient basis while 25 (30%) patients needed to be admitted, including one admission to the intensive care unit (ICU). Only 14 (29%) patients in the falciparum group were managed as outpatients and 34 (71%) patients required admission. The mean duration of admission was 3.96 days (range 1–10 days) in the vivax group and 5.56 days (range 1–20 days) in the falciparum group, with one death in each of these groups. Both the patients who died presented with shock and multiorgan dysfunction (acute adult respiratory distress syndrome, acidosis, severe thrombocytopenia, hepatic and renal dysfunction). These patients had SOFA scores of 18 and 19; and both required invasive ventilation and inotropic supports.

DISCUSSION

In this prospective clinico-epidemiological hospital based study of 131 cases of malaria over one year period, *P. vivax* remains the most common infecting malarial species (63%) followed by *P. falciparum* (27%) while minority of the cases (10%) were due to dual infection. The mean age of the patients in the study was 33 yr [Interquartile range (IQR) 15–76 yr] and the distribution of the age across all the three groups was similar to other studies reported from India[9-12]. There is a significant male preponderance (85% overall) across all the groups. Although, WHO describes the people with comorbid conditions are "at risk" to develop severe vivax malaria, but our findings indicates that severe vivax malaria remains the commonest causative species and infects younger males without comorbidities.

Thrombocytopenia, the most common hematological abnormality, was observed in all the three groups and is similar to some of the earlier studies[13–16]. In the present study, it was established that the proportion of patients with severe thrombocytopenia or overt bleeding manifestation in vivax group is comparable to the severity observed in the falciparum or mixed infection group. Although the mean hemoglobin level was significantly lower in the falciparum group and a trend towards significance was observed in the mixed infection group as compared to vivax monoinfection, yet the clinically relevant severe anemia (Hb < 5 g/dl) was not statistically significant. The white blood cell counts were not statistically different across the three groups of malaria (Table 2). These results clearly indicate that vivax malaria can cause similar degree of hematological complications as compared to falciparum malaria without any significant increase in clinical bleeding[17].

The proportion of patients with clinical jaundice and the bilirubin level was not significantly different between the vivax and falciparum group. Compared to the studies from Karnataka, Maharashtra (India) and Pakistan there

exists considerable variations in the degree and proportion of patients with hyperbilirubinemia[5, 11, 13, 18-19]. Variation in the total serum protein and transaminases achieved statistical significance among the three groups but the clinical relevance of these findings in terms of either grading of severity or indicator of mortality could not be ascertained. Since the level of parasitaemia in case of *vivax* is not routinely done in our laboratory, so the correlation of degree of hyperbilirubinemia with the parasitic index was not possible. These findings reiterate that *vivax* malaria can cause significant hepatic dysfunction.

The incidence of acute renal failure in the vivax group was similar to that in the falciparum group but the proportion of patients with serum bicarbonate level < 15 mEq/dl was significantly higher in the falciparum group. On review of the literature, it was observed that, within Indian studies, there is considerable variation in the incidence of renal failure in *vivax* malaria as reported in the studies from Mumbai (32%), Manipal (5%) and Aligarh (2%)[10, 18-19]. Serum urea level, as a marker of acute decline in glomerular filtration rate, was found to be significantly higher in the falciparum group than vivax group. This the first study from India to compare urea and bicarbonate levels among the prominent malarial species.

The occurrence of CNS, cardiopulmonary complications and mortality in patients infected with different malarial parasites in the vivax group was statistically similar in proportions to that of falciparum or mixed infection. From this comparative study, we highlight that previously deemed "benign" vivax malaria may not be essentially true and can be no longer considered uncomplicated. The early diagnosis and prompt initiation of antimalarial therapy and resuscitative measures can decrease the morbidity and mortality due to *P. vivax*[19].

CONCLUSION

In conclusion, *P. vivax* is emerging as an important cause of malarial morbidity and mortality and all the complications of falciparum malaria may be seen in vivax malaria as well. To date, most studies on pathogenesis, preventive strategies and treatment of malaria have focused on falciparum malaria due to its presumed exclusivity in causing severe malaria. However, with increasing evidence to the contrary, there is a pressing need to study the issues related to severe vivax malaria in order to advance the efforts for elimination of this disease.

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