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Cross Sectional Study of Severe and Mixed Malaria Infections : Experience of a Tertiary Care Hospital in South-West Coastal Karnataka

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Abstract

Background: India launched the National Framework for Malaria Elimination to encourage surveillance and strategies towards 'elimination' rather than control by 2030. Considering the significant challenges on this path, regional variations in clinical and haematological manifestations are useful parameters to help shape national elimination strategies. Our study aims to compare the demographic, clinical and haematological parameters among severe malaria cases and highlight mixed malaria infection.

Methods: A cross sectional study was carried out between January 2015 and May 2018. Diagnosis was done by peripheral smear microscopy, followed by immunochromatographicrapid test and finally quantitative buffy coat test. Patients were classified as severe and non-severe disease according to WHO major criteria. The relevant data of the study subjects was collected from inpatient case records and analysed.

Results: A total of 403 inpatients with confirmed malaria were included in our study. Severe malaria was observed in 21.5% and these patients had a significantly longer stay in hospital of 6.08 ± 3.78 days. Infections caused by P. falciparum (48.6%) and P. vivax (46.9%) were almost equal in number. Acute respiratory distress syndrome was observed more in 21.4% (9/42) of P. vivax infections. Mixed malarial infections were observed in 4.5% (18/403) of total patients and 33% of mixed malarial cases presented with severe manifestations.

Conclusions: Attributing severe malaria to P. falciparum or P. vivax alone can be misleading especially in regions with complicated epidemiology, like India. Identification of malarial coinfectionsinfection are difficult without molecular diagnostic tools. Incorrect diagnosis may directly affect appropriate antimalarial therapy selection.

Highlights

- Clinical and haematological manifestations have not been observed as a malaria burden metric in all regions.
- Identification of malarial coinfectionsinfection are difficult without molecular diagnostic tools.
- Appropriate laboratory diagnosis of mixed malarial infections aids in selecting antimalarial therapy.
- Study of regional variations in malaria presentations can improve public health.

Keywords: Malaria co-infection; Plasmodium; India; Endemic; Eradication.

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Background

Malaria remains a significant global health problem with 228 million cases worldwide in 2018[1]. India accounts for 4% of the globally reported cases and reports 70% of total malarial cases in south east Asia due to the endemicity of plasmodium parasites to this region[2]. Global co-operative efforts to reduce the malaria burden have allowed hyperendemic regions such as India to report a 24% decrease

since 2016[3]. This reduction in cases is credited to the national malaria control program (NMCP) and the national vector borne disease control program (NVBDCP) launched by the government of India. However, despite significant national efforts, malaria continues to make up one of the common inpatient diagnoses in India.

Malaria is highly endemic and persistent throughout the year in several parts of southwestern regions of India, including a substantial portion of Karnataka state. Of the 27 districts, Udupiharbours high vector density contributing to the high case incidences (0.1-1 confirmed cases per 1000 population) in this region.[1] The NVBDCP identified Udupi as the second highest district reporting 1028 cases of malaria from April 2016 to March 2017[4]. Considering the high endemicity and huge health burden, additional reports maybe required on demographic, clinical and hematological factors that contribute to severe malaria manifestations in coastal tropical regions like Udupi. As malaria is a dynamic disease that disproportionately affects the rural poor, its epidemiology is affected by construction, poverty, and changing global climate; making continual investigation necessary [5]. Therefore, in line with global malaria eradication goals by 2030, it is useful to assess potential changes in the distribution, presentation and severity of malaria to prepare for future malaria-specific interventions[5–8]. The present study aims to compare the demographic details, clinical features, haematological parameters, complications among severe malaria; as well as highlight mixed malaria infection.

Methodology

2.1 Study design &population :A retrospective cross sectional timebound study was conducted following approval by Institutional Ethics Committee, IEC No.–672/2019. Demographic, clinical and laboratory data from inpatients with laboratory diagnosis of malaria for 3.5 years, between January 2015 and June 2018 admitted for > 48 hours was collected in pre-designed detailed case record forms. Outpatients being treated for malaria were excluded from study. On the basis of clinical presentation, inpatients were then classified as severe and non-severe malaria according to WHO major criteria and further analysed.

2.2 Study definitions : Criteria for classification of plasmodium infection as severe malaria is defined by WHO; including dangerous manifestations like thrombocytopenia, pulmonary edema, respiratory distress syndrome and impaired consciousness (See additional file 1).[9,10] Patients with coinfection with P. falciparum and P. vivax were laboratory confirmed cases by microscopy and rapid diagnostic test.

2.3 Malaria diagnostics :Diagnosis of patients included in the study was performed on peripheral blood samples by conventional microscopy, followed by rapid diagnostic test (immunochromatographic method) and finally quantitative buffy coat (QBC) test during the study period as per standard guidelines.

2.5 Peripheral smear : Thin smears were examined according to WHO recommendations.[1] Thin smears were made to identify malaria species (including the diagnosis of mixed infections), quantify parasitaemia, and assess for the presence of schizonts, gametocytes. Negative result have been reported after screening at least 200 OI visual fields under 1000x magnification. The level of parasitaemia may be expressed either as a percentage of parasitized erythrocytes or as the number of parasites per microliter of blood, however was not recorded in present study.

2.6 Rapid diagnostic test :MalariaP.f/P.v antigen (SD BIOLINE, Abott, California, USA) was used to detect circulating parasite antigens targeting histidine-rich protein-2 (HRP-II) for P. falciparum and lactate dehydrogenase for P. vivax (pLDH). The test was performed using 5µl of anticoagulated venous blood; the sample was added to specimen well and 4 drops of assay diluent was added to square well. The test was interpreted as per the manufacturer's instructions after 15 min. It was recorded as positive for P. falciparum or P. vivax if P.f and P.v bands respectively were seen, along with control (C) band.[11]

2.7 Quantitative buffy coat test : QBC technique (Kapillery, FlorotekBiosystems, Mumbai, India) was used for the detection of malarial parasites in blood using microhematocrit tubes coated with acridine orange 65µl of blood and stopper and float were added at either ends into the tubes. The tubes were centrifuged at 12000rpm in a pre-programmed centrifuge as per the kit instructions. The visualisation was done using a fluorescent microscope under 100x objective. Parasites were found to have bright green fluorescence at the interface between RBC and buffy coat layer.

Mixed malarial infections were identified as a combination of mixed morphology (P. falciparum and P. vivax) microscopy and rapid diagnostic test with positive P.f along with P.v bands.

2.8 Malaria Management: Patients confirmed to have malaria were treated according to national

recommended guidelines i.e., chloroquine (25mg/kg) for 3 days along with primaquine (0.25mg/kg) for 14 days for vivax malaria. Falciparum malaria treatment was either chloroquine for 3 days along with single dose primaquine on the second day or artesunate–sulfadoxine–pyrimethamine combination therapy (fixed dose of artemisin combination therapy, ACT) for 3 days along with single dose of primaquine on the second day.[3] Any patients admitted for malaria management was based on treating clinician discretion.

2.9 Statistical Analysis: The relevant demographic, clinical and laboratory data of the study subjects was recorded and evaluated with SPSS ver. 16 (SPSS Inc., Chicago, IL, USA). Quantitative data was presented as mean ± SD or median, IQR. Chi-square test was used to test significance between categorical variables. Independent sample t test was used to compare means across two groups. The level of significance for all statistical tests was set at 5% ($p < 0.05$) and the results were reported within 95% confidence interval.

During study period, a total of 403 patients were included in the study and the majority, 340 (84.4%) were male. Almost all patients were clinically stable on discharge, only 1 patient was discharged against medical advice. P. falciparum infection was reported in 196 (48.6%) of the patients and P. vivax infection was found in 189 (46.9%) patients as illustrated in table 1. Severe malarial infections showed a significantly longer stay in hospital at a mean 6.08 ± 3.78 [p <0.001, OR 0.783 (95% CI 0.703 – 0.873)].

3.2 Characteristics of severe malarial infection caused by P. falciparum and P. vivax.

Severe malaria, classified according to WHO (2014) has been reported in 87 (21.5%) cases.[9] Mixed malaria cases were excluded and were analysed separately. Furthermore, majority of severe infections were due to P. vivax(42/82, 51.8%). Clinical and laboratory criteria have been tabulated in table 2. Interestingly, previous history of malaria was significantly associated with P. falciparum [p = 0.010, OR 2.273 (95% CI 1.76 – 2.93)].

Clinical presentations of severe infections were analysed and patients presenting with acute respiratory distress were observed to be significantly more in P. vivax infections [p = 0.010; OR 2.273 (95% CI 1.76 – 2.93)]. Among haematological parameters, MCHC was found to be significantly lower in P. falciparum when compared to P. vivax. No derangement of blood parameters were significantly associated with P. falciparum or P. vivax infection. 3.3 Sub group analysis for patients with mixed malarial infection.

Results

3.1 Demographic and clinical Characteristics of study population.

Table 1: Demographics, comorbidities and other patient characteristics (N= 403) (%).

	Overall	Severe	Non Severe	P value
Age (years; mean ± SD)	35.8 ± 14.86	37.2 ± 15.09	35.5 ± 14.80	-
Male sex (n,%)	340 (84.4)	69 (79.3)	271 (85.8)	-
Origin (n,%)				
Udupi	245 (60.7)	47 (54)	198 (62.7)	-
Karnataka	117 (29)	34 (39.1)	83 (26.3)	-
Other states	41 (10.1)	6 (6.9)	35 (11.1)	-
Comorbid conditions (n,%)				
Type 2 diabetes mellitus	9 (2.2)	3 (3.4)	6 (1.9)	0.413
Chronic renal dysfunction	3 (0.7)	1 (1.1)	2 (0.6)	0.519
Carcinoma/ metastasis	3 (0.7)	2 (2.3)	1 (0.3)	0.119
Previous history of malaria	27 (6.7)	7 (8.0)	20 (6.3)	0.571
Plasmodium infection (n,%)				
P. falciparum	196 (48.6)	39 (44.8)	157 (49.7)	-
P. vivax	189 (46.9)	42 (48.3)	147 (46.5)	-
Mixed Infection	18 (4.5)	6 (6.9)	12 (3.8)	-
Duration of Stay in hospital (days; mean ± SD)	4.8 ± 2.42	6.08 ± 3.78	4.53 ± 1.74	<0.001*
Duration of fever (days; median, IQR)	5.6 (3,7)	5 (3,7)	4 (3,6)	0.024*
Improved outcome (n,%)	402 (99.7)	86 (98.8)	316 (100)	-
Total	403	87	316	

Table 2: Clinical and laboratory characteristics in severe malaria N = 81.(%)

	P. falciparum	P. vivax	p
Age (years; mean ± SD)	35 ± 14.8	36.5 ± 15.8	-
Male sex (n,%)	30 (76.9)	34 (81)	0.656
Duration of Stay in hospital (days; median, IQR)	5 (4, 8)	5 (3.7, 7)	0.355
Duration of fever (days; median, IQR)	6 (3, 7)	5 (3, 7)	0.299
Previous history of malaria	6 (15.4)	0 (0)	0.010*
Improved outcome (n,%)	39 (100)	42 (100)	-
Severe anaemia (Hct<15% or Hb<5 mg/dL)	21 (53.8)	19 (45.2)	0.508
Renal impairment (Urine output<400ml/ 24 hours and S. Creatinine >3mg/dL despite adequate volume repletion)	0 (0)	1 (2.4)	1
Hypoglycaemia (RBS < 40 mg/dL)	0 (0)	3 (7.5)	0.242
Jaundice (bilirubin> 3 mg/ dL; n,%)	18 (46.2)	21 (50)	0.729
Hepatomegaly (n, %)	13 (33.3)	9 (21.4)	0.229
Splenomegaly (n, %)	10 (25.6)	6 (14.3)	0.2
Impaired consciousness (rousable mental condition)	4 (10.3)	6 (14.3)	0.739
Repeated generalised convulsions (>3 convulsions within 24 hours; n, %)	0 (0)	1 (2.4)	1
Pulmonary Edema (Radiological evidence and lung injury score; n, %)	1 (2.6)	6 (14.3)	0.110
Acute respiratory distress syndrome (Respiratory rate >30/min or O2 saturation < 92%; n,%)	2 (5.1)	9 (21.4)	0.05*
Circulatory collapse (algid malaria) (SBP <70 mmHg; n, %)	1 (2.6)	5 (11.9)	0.203
Leukopenia (<4000/µL; n,%)	6 (15.4)	9 (21.4)	0.484
Leucocytosis (>10,000/µL; n,%)	2 (5.1)	3 (7.1)	1
Absolute neutrophil count (109/µl; median, IQR)	2.8 (1.2, 6.8)	2.9 (1.5, 4.9)	0.833
MCV (fl; median, IQR)	82.8 (80.4, 92.6)	85.1 (81.6, 91.5)	0.359
MCH (pg/cell; median, IQR)	28.1 (26.6, 30.2)	29 (27.9, 31)	0.097
MCHC (g/dL; median, IQR)	33.1 (32, 34.5)	33.8 (33.4, 34.5)	0.006*
RBC (1012/L; median, IQR)	3.8 (3.0, 4.7)	4.1 (3.5, 4.7)	0.312
Platelets (104/L; median, IQR)	6 (3.3, 13.2)	5.8 (3.0, 8.9)	0.419
S. Potassium (mmol/L; median, IQR)	4.2 (3.8, 4.6)	4.1 (3.8, 4.5)	0.412
Plateletcrit(/µl; median, IQR)	0.05 (0.03, 0.12)	0.05 (0.02, 0.09)	0.172
Total	39	42	

*p<0.05, Statistically significant.

Coinfection with P. falciparum and P. vivaxwas diagnosed in 4.5% (18/403) of study population,as presented in table 3. All patients with mixed infection had an improved outcome. No cases with history of acute renal impairment, G6PD or hypotension (systolic BP <80mmHg) were identified.Majority of cases (72.2%) were managed by artesunate–sulfadoxine–pyrimethaminealong with primaquine after microscopic confirmation of diagnosis.

Discussion

India and other south east Asian countries have committed to eliminate malaria by 2030.[3] This is a challenging goal as P. falciparum and P. vivax burden varies across India.[12,13] The state of Karnataka reports an annual parasite index of 1-2/1000 population for P. vivax and a parasite index of <1/1000 population for P. falciparum.[13]

Table 3: Demographics, comorbidities and other characteristics among patients with mixed malaria infection. (N = 18)

	N (%)
Age (years, mean ± SD)	38.8 ± 15.12
Males : Females	3.5:1
Duration of Stay in hospital (days, mean ± SD)	4.6 ± 2.09
Severe Malaria (n, %)	6 (33)
Previous history of malaria (n, %)	1 (5.6)
Improved Outcome (n, %)	18 (100)
Clinical features	
Duration of fever (days; median, IQR)	5 (3.7, 8.5)
Anemia (Hb<7 mg/dL; n,%)	5 (27.8)
Jaundice (bilirubin > 3 mg/dL; n, %)	1 (5.5)
Hepatomegaly (n, %)	4 (22.2)
Splenomegaly (n, %)	3 (16.6)
Laboratory parameters	
Hb (mg/dL)	11.6 ± 1.9
WBC (x 10 3 /uL; median, IQR)	5500 (3700,6200)
Random Blood Sugar (mg/dL)	153 ± 59.2
Absolute neutrophil count (/µl; median, IQR)	2.8 ± 0.76
MCV (fl; median, IQR)	83.1 ± 7.34
MCH (pg/cell; median, IQR)	27.8 ± 2.67
MCHC (g/dL; median, IQR)	33.3 ± 0.87
RBC (/µl; median, IQR)	4.08 ± 0.47
Platelets (x103; median, IQR)	6.2 (3.3, 8.9)
S. Potassium (mmol/L; median, IQR)	4.08 ± 0.61
Plateletcrit(/µl; median, IQR)	0.06 (0.3, 0.8)
Choice of management	
Artesunate + Primaquine(n, %)	13 (72.2%)
Quinine + Primaquine + Artemether + Lumefantrin (n, %)	1 (5.6%)
Artesunate + Primaquine + Artemether + Lumefantrin (n, %)	2 (11.1%)
Chloroquine + Primaquine (n, %)	2 (11.1%)

Although malaria control measures impact both P. falciparum and P. vivax malaria, P. vivax remains difficult to control in urban areas due to complex epidemiology and clinical presentations.[13] In present study, hospital admission details of severe malaria patients were analysed; severe cases were admitted for a significantly longer duration of 6.08 ± 3.78 days ($p < 0.001$). These cases also had a longer duration of fever ($p < 0.024$) on admission when compared to patients presenting with non-severe malaria.

The above mentioned factors may be explained by higher parasitaemia usually seen in patients presenting with severe malaria, regardless of the infecting species.

P. falciparum attributed to 48.6% (196/403) of malaria cases and 46.9% (189/403) to P. vivax. Mixed infections were observed in 4.5% (18/403) patients during the study period. These results corroborate the reports of shift in the dominant infecting Plasmodium species in India, from P. vivax to P. falciparum.[14] However there are large regional differences in the epidemiology of malaria in India. An apparent increase in cases of falciparum malaria in present study can be attributed to the fact that i) such patients require frequent hospitalisation, ii) monovalent RDTs are used for diagnosis in the primary care setup and iii) that mixed infections showed a slightly more pronounced manifestation than P. vivax malaria, as seen in some other studies.[15,16] Interestingly, on analyzing severe cases, the majority were attributed to P. vivax accounting for 48.3% (42/87). This observation has been reported over the years from endemic regions of vivax malaria.[17] Maguire JD et. al. have highlighted and have cautioned against severe forms of vivaxmalaria in tropical areas such as our study region.[18,19] On analysis of previous admissions, repeat infection of an unknown malarial parasite in a 6 month interval were more likely to be found in P. falciparum [$p = 0.010$; OR 2.273 (95% CI 1.76 – 2.93)]. This suggests that there may be either inadequate management of the initial infection at the primary healthcare centre or repeat infection due to high vector burden in study region. This finding may also be due to some amount of misdiagnoses of mixed malarial infections at the primary level due to dependency on microscopy and RDTs.[14] On analysis of severe malaria cases, severe anaemia followed by jaundice were the most common presentations irrespective of infecting species. Significant association between acute respiratory distress syndrome and P. vivax infections was observed [$p = 0.05$; OR 0.198 (95% CI 0.04 – 0.98)].

Respiratory dysfunction has been reported from other studies done from similar study regions, however its association with P. vivax is unclear.[7] Val et. al. suggested that similar to P. falciparum infection, P. vivax may also demonstrate sequestration of parasitized red blood cells in the pulmonary vasculature.[20] No cases of severe thrombocytopenia, disseminated intravascular coagulation (DIC) or abnormal bleeding were observed in present study. These findings differ from the most prevalent severity signs associated with vivax malaria including, severe thrombocytopenia, Circulatory collapse/ shock and hepatic dysfunction.[21] Some clinical characteristics, comorbidities and manifestations as

mentioned in the patient case files, e.g., impaired consciousness without continued Glasgow Coma Scale (GCS) monitoring and hypotension without evidence of decreased perfusion, may have caused an overestimation or underestimation of severe malaria and remains a limitation of present study.

Furthermore, parasitemia levels in study population are lacking as only reports of >10,000 parasites/µl have been noted in patient medical records with specific request from admitting physicians. In fact, the lack of data on outpatient malaria contributes to an inaccurate presentation of the overall patients with confirmed malaria diagnosis. Differences in severity presentations with respect to suspected infecting Plasmodium spp. are important for the management and diagnosis of malaria. The above observations suggest that WHO definitions, largely based on severe falciparum malaria, may require revisions to include varying clinical presentations of vivax or mixed malarial infections.[7,9] Comparing of laboratory parameters, leukopenia or leucocytosis was not found to be significantly associated with severe vivax malaria ($p = 0.484$, $p = 1$), this finding is comparable to a study by Kotepui et. al. where only patients with very high parasitaemia (>10 parasites/oil field) tended to have higher leukocyte counts.[16] Our study found no significant associations between haematological parameters of severe malaria on the basis of infecting Plasmodium spp.

A subgroup analysis was performed for 18 (4.5%) patients with mixed P. falciparum and P. vivaxinfection. Mixed malarial infections have been reported in up to 13% of total infections with an increased prevalence in middle and southwest coast of India.[14] Majority of the patients were young, 38.8 ± 15.12 years of age and were male (77.8%). This demographic is considered the major contributor to the Indian workforce. Patients were admitted for an average of 4.6 ± 2 days, which highlights the necessity of prolonged inpatient stay for patients with mixed infections. In our study, mixed malaria infection were not confirmed using molecular methods, and diagnosis was based on microscopy and rapid assays. This has shown to underestimate the burden of mixed infection in endemic regions.[7,14] The sensitivity, when compared to PCR, of RDTs when diagnosing mixed malarial infections is 58.3% and with microscopy is only 16.6%.[22] RDTs in India are limited by poor sensitivity and future implementation of molecular techniques are imperative for accurate diagnosis of mixed infections. Rise in reports of mixed malarial infections have also been linked to changing climate conditions along with increasing migration, urbanisation and globalisation in tropical regions.[23]

Therefore, diagnosis and appropriate management for mixed malarial infections are paramount when considering elimination goals. Furthermore, the chance of recurrence in mixed malaria cases due to P. vivax must be considered when implementing appropriate treatment and control measures. Majority of mixed malarial infection were managed with artemisinin derivatives or quinine along with primaquine. There were no fatalities reported in study population. Our study suggests, attributing severe malaria to P. falciparum or P. vivax alone can be misleading especially in regions with complicated epidemiology, like India. Furthermore, in mixed malarial infection diagnosis, presentation and management requires multidisciplinary attention from clinical, laboratory and community perspectives. Research addressing clinical and laboratory manifestations in malaria endemic zones remain essential to maintaining a robust and validated database to help establish clear recommendations for malaria elimination.

Conclusion

Malaria continues to make up a significant partof the inpatient diagnosis in India and activities towards elimination are hindered by the complex epidemiology in endemic regions like coastal Karnataka. Attributing severe malaria to P. falciparum or P. vivax alone can be misleading especially in such regions. Furthermore, identification of malarial coinfections are difficult without molecular diagnostic tools. Laboratory aid in diagnosing mixed malarial infections is paramount in selecting appropriate antimalarial therapy and ensuring health and safety of the patient.

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