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A prospective study on adult patients of severe malaria caused by *Plasmodium falciparum*, *Plasmodium vivax* and mixed infection from Bikaner, northwest India

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ABSTRACT

Background & objectives: Description of severe vivax malaria and mixed species infection requires good clinical study. The present study was undertaken to evalute the characteristics of severe malaria patients in Bikaner, northwest India.

Methods: This prospective study included 539 admitted adult patients of severe malaria (*Plasmodium falciparum* 274, *P. vivax* 221, and mixed infection of *Pv* + *Pf* 44). The diagnosis was confirmed by polymerase chain reaction. The categorization of severe malaria was done strictly as per WHO criteria.

Results: The distribution of severe manifestation was similar in severe vivax, falciparum and mixed infections except more cases of thrombocytopenia in *P. vivax* (p=0.030) and in mixed infection (p=0.004). The risk of developing severe malaria was greatest in patients of mixed infection [53.01% (44/83)] in comparison to *Plasmodium falciparum* malaria [49.37% (274/555), RR= 1.135; p=0.616] and *P. vivax* malaria [45.38% (221/487), RR = 1.299, p=0.243]. Hepatic dysfunction was the commonest pernicious syndrome [*P. falciparum* 50% (137/274), *P. vivax* 43.89% (97/221), and mixed infections 54.55% (24/44)]. Multiorgan dysfunction was present in 40.26% (217/539) patients, the risk was greatest in mixed infection [90.90% (40/44)] in comparison to *P. falciparum* monoinfection [37.59% (103/274), RR = 12.238; p=0.0001] or *P. vivax* monoinfection [33.48% (74/221), RR = 13.25; p=0.0001]. The risk of mortality in severe malaria was 6.31% (34/539) in which mixed infection had greater risk [9.09% (4/44)] in comparison to *P. falciparum* [7.30% (20/274); OR = 1.270 (CI 0.347–4.217); p=0.757] or *P. vivax* [4.52% (10/221); 0R 2.110 (CI 0.527–7.826); p=0.260].

Interpretation & conclusion: Severe vivax or falciparum malaria had almost similar features and prognosis including mortality. Risk of developing severe malaria, multiorgan dysfunction and mortality was more in patients of mixed infection in comparison to *P. falciparum* or *P. vivax* monoinfection. A multicentric study on larger number of patients requires further confirmation.

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

INTRODUCTION

Plasmodium vivax malaria is found across a large area of the globe and potentially affects larger number of people than *P. falciparum* malaria. It is endemic across around 44 million km^2, approximately a third of earthland. India alone contributes nearly half (46%) of the global population at risk and two thirds of those are at stable risk[1]. In spite of this, most of the research and published literature on malaria focus on *P. falciparum* and the *P. vivax* remains a neglected tropical disease[2]. However, last few years have witnessed a major shift in relation to research on clinical and epidemiological behaviour of *P. vivax*[3–4]. Firstly, there are increasing reports in literature from different parts of the world through different types of studies describing severe and sometime fatal malaria including deaths by *P. vivax* in certain settings[5–31]. Reported severe manifestations include cerebral malaria, generalized convulsion and status epilepticus, hepatic dysfunction and jaundice, acute lung injury, acute respiratory distress syndrome (ARDS) and pulmonary edema, acute kidney injury, severe anaemia, severe thrombocytopenia with or without bleeding, hypoglycemia and shock[6,12,14]. Recently, the deaths due to *P. vivax* monoinfection have also been established by histopathological confirmation of autopsy specimens[32–33]. In some of these studies, the diagnosis of *P. vivax* monoinfection was established by polymerase chain reaction (PCR) examination thereby negating any chance of coinfection with *P. falciparum*[5,6,9,12–15,17–19,28] and have undertaken exhaus-

tive clinical, biochemical and radiological studies to rule out associated comorbid conditions[6,12–14,17–18,28].

The second puzzling question is in relation to the clinical profile of mixed (*P. vivax* with *P. falciparum*) infection. There are various reports in literature providing evidence of increasing/no change[7, 11] or reducing[34–37] the severity and morbidity in patients of mixed infection in relation to *P. falciparum* monoinfection. However, the diagnosis in these patients was not confirmed by PCR examination thereby limiting the reliability of exact speciation in comparison to molecular diagnosis[16–17].

The characteristics and outcome of severe malaria are best assessed through a prospective study in an ethnically homogenous sample and with clinical and laboratory data sufficient to allow accurate diagnosis as well as the detection of important comorbid condition[17]. Many of the earlier studies in relation to the description of severe *P. vivax* malaria and severe mixed infection malaria have neither used strictly defined WHO criteria nor ruled out associated comorbid conditions. In majority of these studies, the diagnosis was established by peripheral blood film (PBF) examination only thereby not providing enough strength in clinical and species diagnosis[7, 10–11]. Earlier, we have carried out hospital-based prospective observational study to describe the clinical presentation of PCR confirmed cases of severe vivax malaria in children and adults[6,9,14,18]. In this prospective hospital-based observational study on adult patients of PCR diagnosed severe malaria (*P. falciparum*, *P. vivax* and mixed infection), in which associated comorbid conditions were ruled out by thorough laboratory evaluation, we have carried out detailed clinical, biochemical and radiological examinations to study the complete clinical spectrum of severe illness as described by WHO for *P. falciparum* malaria[38] along with thrombocytopenia as well as to study the effect of mixed infection on morbidity and mortality.

MATERIAL & METHODS

Study site

This prospective study was carried out at the Department of Medicine, Sardar Patel Medical College and associated group of Hospitals, Bikaner, Rajasthan, India from January 2007 to December 2008. Bikaner is a part of Thar Desert and is hypoendemic region for malaria. Hospital guideline requires detailed study of PBF examination for malaria parasites for all the patients presenting with history of fever.

Study procedures

This prospective study was conducted on admitted adult patients of malaria in whom the diagnosis was done by PBF and rapid diagnostic test (RDT). After thorough clinical and laboratory examination, the categorization of severe malaria and treatment was done according to WHO guidelines[38]. The final confirmation of species diagnosis was done by PCR examination. The study plan was approved by the hospital research committee and a written consent of patients/relatives was mandatory. Further, details of all the patients were collected on a study proforma (used in our earlier studies also)[6,12,14] by a study team of researchers.

Selection criteria

Adult patients of malaria with severe manifestations and evidence of asexual phase of malaria parasite in PBF and/or positive RDT along with positive PCR evidence of malaria.

Exclusion criteria

Patients who refused to give the written consent or had evidence of other concurrent illness were not included in the study.

Laboratory procedures

Diagnostic methods used for detection of malaria parasites were conventional thick and thin PBF stained with Giemsa stain and microscopically examined under oil immersion. The slide was considered negative when there were no parasites in the 200 high-power field. The RDTs were based on detection of specific *Plasmodium* antigen, lactate dehydrogenase (OptiMal test; Diamed AG, Cressier sur Morat, Switzerland) and histidine-rich protein-2 (Falcivax test; Zephyr Biomedical System, Goa, India). Final categorization of *P. vivax*, *P. falciparum* or mixed infection was done by PCR examination in all the patients having severe manifestations. Other laboratory investigations in all the patients of severe malaria included complete blood count, platelet count, bleeding time, clotting time, blood glucose, blood urea, serum creatinine, serum bilirubin (conjugated and unconjugated), serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum alkaline phosphatase, complete urine analysis, electrocardiogram, and appropriate blood test to rule out typhoid fever (typhi dot test), leptospirosis, and dengue infection (differential detection of IgG and IgM antibodies) and HIV. Depending upon the clinical situation, other tests included skigram chest, serum electrolytes, and arterial blood gas analysis for acute respiratory distress syndrome (ARDS); fundus examination, cerebrospinal fluid (CSF) examination, computerized tomography (CT) of the head and

electroencephalography (EEG) in patients having repeated convulsion and cerebral malaria (CM); ultrasonography of whole abdomen and specific test for hepatitis B and C in hepatic dysfunction and jaundice and glucose-6-phosphate dehydrogenase (G6PD) enzyme level (kinetic method: G-SIX Kit, Crest Biosystems, Goa) for hemolysis. Blood culture was taken on brain-heart infusion broth in every patient who was having continuous high grade fever >101°F for > 24 h after admission. Parasite density was estimated in all the patients of severe vivax malaria. The PCR confirmation was done in all the patients having severe manifestations with evidence of malaria on PBF and/or RDT. The PCR studies were targeted against the 18S ribosomal RNA gene of the parasite and used one genus specific 5′ primer and two species-specific 3′ primers in the same reaction mixture and details are described in earlier reports[6,12,14]. All the clinical syndromes were classified according to WHO[38] criteria and the involvement of two or more than two organs were considered as multiorgan dysfunction (MODS). Specific antimalarial treatment was given in the hospital according to WHO guidelines[38].

Statistical analysis

Morbidity spectrum and mortality pattern of patients infected with *P. falciparum* monoinfection, *P. vivax* monoinfection and mixed infection were compared and analyzed individually with Odds ratio with 95% confidence interval *via* SPSS version 14.0.

RESULTS

During the study period (January 2007 to December 2008), 24,703 adult patients of fever attended the hospital, of which 1155 patients had positive evidence of malaria by PBF examination and/or RDT test and required admission. Exact species diagnosis of severe malaria was confirmed by PCR examination. A total of 32 patients who had evidence of concomitant illness, unable to get written consent and had problems related to confirmation of species by PCR examination, were not included in the study. Thus, the subsequent analysis was done in 1123 patients, that included 584 uncomplicated malaria and 539 severe malaria patients. The details of species diagnosis along with distribution of complicated and uncomplicated

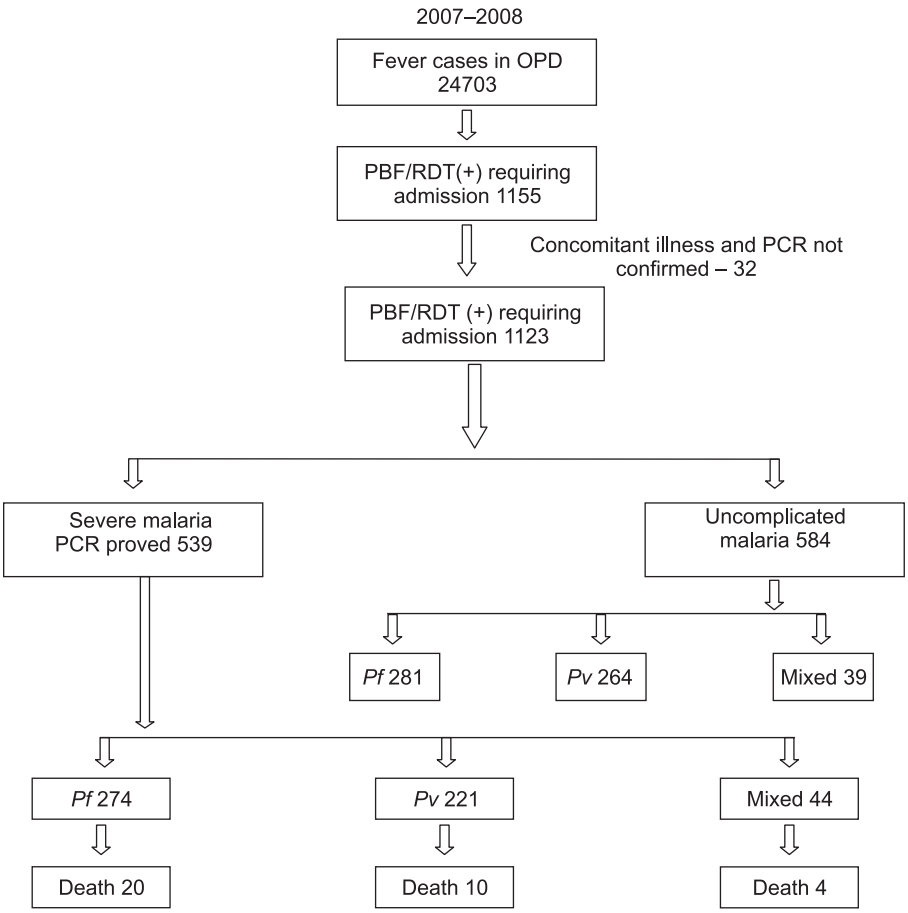

Fig. 1: Flow chart showing different stages for final categorization of severe malaria.

Table 1. The distribution of pernicious syndrome (n=539) in different species

Malaria species Complications	*P. falciparum*		*P. vivax*		Mixed infection		Comparison group	95% Confidence interval (CI)			
	(n=274)	%	(n=221)	%	(n=44)	%		Odds ratio	LL	UL	*p*-value
Cerebral malaria	29	10.58	21	9.50	5	11.36	*Pv vs Pf*	0.887	0.494	1.593	0.765
							Pv vs mixed	0.819	0.300	2.219	0.781
							Pf vs mixed	0.923	0.347	2.441	0.797
Anaemia	115	41.97	76	34.39	17	38.64	*Pv vs Pf*	0.725	0.502	1.045	0.095
							Pv vs mixed	0.832	0.430	1.610	0.607
							Pf vs mixed	1.149	0.602	2.189	0.743
Thrombocytopenia	86	31.39	91	41.18	24	54.55	*Pv vs Pf*	1.530	1.058	2.214	0.030*
							Pv vs mixed	0.583	0.306	1.111	0.133
							Pf vs mixed	0.381	0.201	0.722	0.004*
Jaundice	137	50	97	43.89	25	56.82	*Pv vs Pf*	0.782	0.548	1.116	0.205
							Pv vs mixed	0.595	0.312	1.135	0.137
							Pf vs mixed	0.760	0.403	1.434	0.421
Renal failure	36	13.14	21	9.50	6	13.64	*Pv vs Pf*	0.694	0.395	1.221	0.257
							Pv vs mixed	0.665	0.258	1.705	0.415
							Pf vs mixed	0.958	0.387	2.361	1
ARDS	2	0.72	2	0.90	0	0	*Pv vs Pf*	1.242	0.217	7.097	1
							Pv vs mixed	–	–	–	–
							Pf vs mixed	–	–	–	–

*Statistically significant; LL— Lower limit; UL— Upper limit.

malaria are shown in Fig. 1. The male : female ratio in patients infected with *P. falciparum*, *P. vivax* and mixed infection was 1.52 (335 : 220), 1.50 (291 : 194) and 2.32 (58 : 25), respectively. Although, 50.83% (274/539) of severe malaria cases were caused by *P. falciparum*. The risk of developing severe malaria was greatest in patients of mixed infection [53.01% (44/83)] in comparison to *P. falciparum* malaria [49.37% (274/555), RR = 1.135; *p*=0.616] and *P. vivax* malaria [5.38% (221/487), RR = 1.299; *p*=0.243]. The details of severe manifestations in patients with *P. vivax*, *P. falciparum* and mixed infections are shown in Table 1. The peripheral blood film of patients of severe vivax malaria showed predominantly trophozoites and the density of parasites was 800–68000/mm^3 (mean ± SD = 14000 ± 13285.85).

Presenting clinical features

Of 274 patients with severe *P. falciparum* monoinfection, 137 (50%) had jaundice, 115 (41.97%) had severe anaemia (haemoglobin <5 g/dl), 86 (31.39%) had thrombocytopenia, 36 (13.14%) had renal failure, 29 (10.58%) had cerebral malaria and 2 (0.72%) had ARDS (Table 1). Patients with severe *P. vivax* monoinfection (n=221) also had the similar phenotypic features reported in severe *P. falciparum* monoinfection without having statistical significant difference except more patients with thrombocytopenia (*p*=0.030). When compared to those with severe *P. falciparum* malaria, patients with mixed infection also had similar phenotype but thrombocytopenia was more common (*p*=0.004) (Table 1). Hepatic dysfunction was the commonest observation in malaria caused by all the species [*P. falciparum* 50% (137/274), *P. vivax* 43.89% (97/221), and mixed infections 54.55% (24/44)].

Multiorgan dysfunction

Multiorgan dysfunction (involvement of two or more organs) was present in 40.26% (217/539) patients having severe malaria. Although, 47.47% (103/217) of multiorgan dysfunction was caused by *P. falciparum* malaria, the risk was greatest in patients having mixed infection [90.90% (40/44)] in comparison to either *P. falciparum* monoinfection [37.59% (103/274), RR = 12.238; *p*=0.0001] or *P. vivax* monoinfection [33.48% (74/221), RR = 13.25; *p*=0.0001]. Potentiality of *P. vivax* malaria for causing multiorgan dysfunction was similar to *P. falciparum* malaria (RR = 1.082; *p*=0.394). The details of different combinations in different species with their statistical significance are given in Table 2. However, the important observation anaemia with jaundice was more common in *P. falciparum* malaria in comparison to *P. vivax* malaria (*p*<0.007) and mixed infection. Anaemia with thrombocytopenia was significantly more common in mixed infection (*p*<0.003) and *P. falciparum* (*p*<0.05) infection in comparison to *P. vivax* infection. Anaemia with cerebral malaria was significantly more

Table 2. Distribution of combination of pernicious syndrome (n=217) in different species

Malaria species Complications	*P. falciparum* (n=274)		*P. vivax* (n=221)		Mixed infection (n=44)		Comparison group	95% Confidence interval (CI)			
	(n=103)	%	(n=74)	%	(n=40)	%		Odds ratio	LL	UL	*p*-value
Anaemia + Jaundice	33	32.04	10	13.51	6	15	*Pv vs Pf*	0.33	0.15	0.72	0.007*
							Pv vs mixed	1.129	0.378	0.579	1
							Pf vs mixed	0.37	0.143	0.97	0.65
Anaemia + Thrombocytopenia	39	37.86	13	17.57	18	45	*Pv vs Pf*	0.349	0.17	0.717	0.05*
							Pv vs mixed	3.83	1.618	9.109	0.003*
							Pf vs mixed	1.342	0.641	2.81	0.55
Anaemia + Cerebral malaria	4	3.88	17	22.97	2	5	*Pv vs Pf*	7.38	2.36	23	0.0002*
							Pv vs mixed	0.172	0.038	0.808	0.02*
							Pf vs mixed	1.302	0.229	7.407	0.5
Anaemia + Renal failure	9	8.74	15	20.27	2	5	*Pv vs PF*	2.65	1.092	6.547	0.04*
							Pv vs mixed	0.207	0.04	0.95	0.05*
							Pf vs mixed	0.549	0.113	2.663	0.35
Jaundice + Thrombocytopenia	35	33.98	24	32.43	16	40	*Pv vs Pf*	0.932	0.492	1.75	1
							Pv vs mixed	0.72	0.324	1.599	0.548
							Pf vs mixed	1.295	0.61	2.749	0.631
Jaundice + Cerebral malaria	6	5.83	6	8.11	6	15	*Pv vs Pf*	1.426	0.441	4.11	0.7
							Pv vs mixed	2	0.599	6.668	0.2
							Pf vs mixed	2.85	0.861	9.445	0.07*
Jaundice + Renal failure	22	21.36	8	10.81	4	10	*Pv vs Pf*	0.446	0.186	1.061	0.1
							Pv vs mixed	0.916	0.258	3.25	0.5
							Pf vs mixed	0.409	0.131	1.273	0.18
Renal failure + Thrombocytopenia	6	5.83	4	5.41	2	5	*Pv vs Pf*	0.925	0.251	3.39	0.5
							Pv vs mixed	0.921	0.161	5.262	0.6
							Pf vs mixed	0.85	0.164	4.407	0.6
Renal failure + Cerebral malaria	4	3.88	4	5.41	2	5	*Pv vs Pf*	1.413	0.342	5.84	0.4
							Pv vs mixed	0.921	0.161	5.262	0.6
							Pf vs mixed	1.3	0.0229	7.401	0.5
Cerebral malaria + Thrombocytopenia	4	3.88	3	4.05	2	5	*Pv vs Pf*	1.045	0.227	4.818	0.6
							Pv vs mixed	1.245	0.199	7.78	0.57
							Pf vs mixed	1.302	0.229	7.4	0.53

*Statistically significant; LL— Lower limit; UL— Upper limit.

common in *P. vivax* malaria in comparison to *P. falciparum* ($p<0.0002$) malaria and mixed infection ($p<0.02$). Anaemia with renal failure was significantly more common in *P. vivax* malaria in comparison to *P. falciparum* malaria ($p<0.04$) and mixed infection ($p<0.05$).

Mortality

Out of 539 patients of severe malaria admitted in the hospital, 34 patients expired (20 *P. falciparum*; 10 *P. vivax*; 4 mixed). There was no fatality in any patient having single organ dysfunction. The risk of mortality in severe malaria was 6.31% (34/539) in which mixed infections had greater risk [9.09% (4/44)] in comparison to monoinfection as a whole [6.06% (30/495); OR = 1.550 (CI 0.439–4.932); $p= 0.511$] and either *P. falciparum* monoinfection alone [7.30% (20/274); OR = 1.270 (CI 0.347–4.217); $p=0.757$] or *P. vivax* monoinfection alone [4.52% (10/221); OR 2.110 (CI 0.527–7.826); $p=0.260$]. The differences were statistically not significant probably because of smaller number of cases in mixed infection malaria group. All the patients had severe manifestations and multiorgan dysfunction. While analyzing the individual severe manifestation, it was found that jaundice was present in majority of cases [85.29% (29/34)] in all types of malaria infections (*P. falciparum* = 90%, *P. vivax* = 80% and mixed infection = 75%). Besides jaundice, other severe manifestations associated with mortality in *P. falciparum* and mixed infection groups were se-

Table 3. Relation of pernicious syndrome and mortality (n=34)

Malaria species Complications	Total (n=34)	*P. falciparum* (n=274)		*P. vivax* (n=221)		Mixed infection (n=44)		Comparison group	95% Confidence interval (CI)			
		(n=20)	%	(n=10)	%	(n=4)	%		Odds ratio	LL	UL	*p*-value
Jaundice	29 (85.29)	18	90	8	80	3	75	*Pv vs Pf*	0.444	0.052	3.738	0.407
								Pv vs mixed	0.75	0.048	11.648	0.670
								Pf vs mixed	0.333	0.022	4.929	0.436
Anaemia	18 (52.94)	13	65	1	10	4	100	*Pv vs Pf*	0.059	0.006	0.574	0.005*
								Pv vs mixed	–	–	–	–
								Pf vs mixed	0.359	0.075	1.714	0.181
Renal failure	16 (47.05)	10	50	4	40	2	50	*Pv vs Pf*	0.667	0.143	3.107	0.449
								Pv vs mixed	1.5	0.145	15.461	0.594
								Pf vs mixed	1	0.116	8.559	0.704
Thrombocytopenia	14 (41.18)	7	35	4	40	3	75	*Pv vs Pf*	1.238	0.259	5.913	0.548
								Pv vs mixed	4.5	0.336	60.15	0.279
								Pf vs mixed	5.571	0.484	64.08	0.177
Cerebral malaria	7 (20.59)	3	15	4	40	–	–	*Pv vs Pf*	3.78	0.648	72.01	0.143
								Pv vs mixed	–	–	–	–
								Pf vs mixed	–	–	–	–
ARDS	2 (5.88)	1	5	1	10	–	–	*Pv vs Pf*	2.111	0.118	37.720	0.563
								Pv vs mixed	–	–	–	–
								Pf vs mixed	–	–	–	–

*Statistically significant; Figures in parentheses indicate percentages; LL— Lower limit; UL— Upper limit.

vere anaemia (*P. falciparum* = 65%, and mixed infection = 75%), renal failure (*P. falciparum* = 50% and mixed infection = 50%) and thrombocytopenia (*P. falciparum* = 35% and mixed infection = 75%) in contrast to *P. vivax* monoinfection in which other associated severe manifestations were cerebral malaria (40%), renal failure (40%) and thrombocytopenia (40%). The details of mortality and different combinations of organ dysfunction with different species are shown in Table 3. The differences were not statistically significant.

DISCUSSION

Severe and complicated malaria is usually caused by *P. falciparum* but it has been increasingly observed that *P. vivax* malaria and mixed infection malaria can also cause similar complication and death in occasional patient. However, most of the studies are lacking in accurate malaria diagnosis by PCR and detection of comorbidities which are likely to influence the clinical course of illness. Moreover, no absolute severity criteria exist for *P. vivax* malaria, however, WHO criteria for *P. falciparum* malaria seems to be applicable and had been used by many previous studies from India, Brazil, Papua New Guinea, Indonesia and other parts of the world. The reported severe manifestations included cerebral malaria, hepatic dysfunction, renal dysfunction, severe anaemia, ARDS, shock, pulmonary edema, haemoglobinuria, hypoglycemia and multiple organ involvement, along with thrombocytopenia which has also been included in many previous similar studies from all over the world[5–31]. In this prospective hospital based clinical observational study on adult patients of malaria, we have carried out detailed clinical, biochemical, radiological and laboratory evaluation of all the patients (*P. falciparum, P. vivax* and mixed infection) to study the complete clinical spectrum of severe illness as described by WHO for *P. falciparum* malaria[38] along with thrombocytopenia. The diagnosis of all the patients was confirmed by PCR to overcome the fallacies in the results obtained by PBF and/or RDT and an attempt was made to be reasonably sure to rule out possibilities of other co-morbid conditions in a scientific manner.

This study included 1123 admitted adult patients, of which 584 were of uncomplicated malaria and 539 were of severe malaria. Although, 50.83% (274/539) of severe malaria was caused by *P. falciparum*, the risk of developing severe malaria was greatest in patients of mixed infections [53.01% (44/83)] in comparison to *P. falciparum* malaria [49.37% (274/555)] and *P. vivax* malaria [45.38% (221/487)]. Patients with severe *vivax* monoinfection (n=221) and severe mixed infection malaria (n=44)) also had the similar phenotypic features as observed in severe *P. falciparum* monoinfection without

having statistical significant difference except thrombocytopenia (Table 1). Hepatic dysfunction was the commonest observation in this study, as was reported earlier in similar studies from India and Brazil[6,12–14,19]. The proportion of patients affected with jaundice (serum bilirubin >3 mg%) was *P. falciparum* 50% (137/274), *P. vivax* 43.89% (97/221), and mixed infections 54.55% (24/44). This finding is similar to the reported observations in severe *P. falciparum* and *P. vivax* malaria in this region[6,12,14]. Renal dysfunction (serum creatinine >3 mg%) was present in 13.14% (36/274), 9.50% (21/221) and 13.64% (6/44) patients having *P. falciparum*, *P. vivax* and mixed infections, respectively. This has also been reported frequently in the Indian subcontinent[6,12,14]. Cerebral malaria (GCS ≤9) was present in 10.58% (29/274), 9.50% (21/221) and 11.36% (5/44) adults having *P. falciparum*, *P. vivax* and mixed infections respectively. Severe anaemia (haemoglobin <5 g/dl) was present in 41.97% (115/274), 34.39% (76/221), and 38.64% (17/44) adults having *P. falciparum*, *P. vivax*, and mixed infections, respectively. Thrombocytopenia (platelet counts <150,000 mm^3) was present in 31.39% (86/274), 41.18% (91/221), and 54.55% (24/44) adults having *P. falciparum*, *P. vivax*, and mixed infections, respectively. Statistically, it was significantly more common in mixed malaria ($p<0.004$) and *P. vivax* malaria in comparison to *P. falciparum* malaria ($p<0.03$). Four patients had ARDS (two each caused by *P. falciparum* and *P. vivax*).

Thus, the observation of this study was that severe *P. vivax* malaria cases have presented with similar phenotypic features to the *P. falciparum* malaria cases except more cases of thrombocytopenia ($p=0.03$). In the patients of mixed infections severe malaria also shared the same phenotypic features of severe *P. vivax* or severe *P. falciparum* monoinfection but the course of illness was more serious, having more patients of multiorgan dysfunction (MODS) and increased mortality in comparison to *P. vivax* or *P. falciparum* monoinfection. Similar observation was also reported by the only other study from Papua New Guinea in which the diagnosis of malaria was confirmed by PCR[17].

During the past few years, reports from Indonesia, Papua New Guinea, India, and the Amazon region describing severe and sometimes fatal disease in *P. vivax* monoinfection have become more frequent. In spite of low level of parasitaemia, the risk approximates those in patients with a primary diagnosis of *P. falciparum* malaria with heavy parasite burden. In a classical review, Baird[39] have provided detailed information of different studies providing enough evidence of morbidity and mortality associates with *P. vivax* malaria. The first detailed study on severe vivax malaria was reported from Bikaner, India describing 11 adult patients, with strong evidence of both sequestration and non-sequestration related complication. Jaundice, ARDS, severe anaemia and renal failure were present in four patients, cerebral malaria and bleeding manifestation in three patients, and shock in two patients. Eight patients had MODS and there was also evidence of pregnancy related complication in the form of premature delivery and death of baby. One patient had post-malaria neurological syndrome (PMNS) in the form of psychosis and death was reported in two patients[6]. Subsequently in 2009, the authors reported 40 more adult cases from the same institution describing similar clinical presentation and MODS[12]. Subsequently in 2010, the authors reported 65 cases of severe *P.vivax* malaria in children and observed that *P. vivax* had more patients of severe anaemia and MODS in comparison to *P. falciparum* and the case fatality rate was almost equal to severe *P. falciparum* malaria (39 from *P. vivax* and 32 from *P. falciparum*)[14]. The important observation from Bikaner region, India was the common evidence of jaundice and renal failure in adults whereas severe anaemia and cerebral malaria were more common in children group[6,12,14]. The only other PCR diagnosed study from central India on 22 cases also reported evidence of cerebral malaria, anaemia, seizure, ARDS and MODS. PMNS was observed in two patients and death was recorded in two patients[28].

The study from Brazil on 17 patients revealed almost similar observation. Ten patients had jaundice, five had anaemia, two each ARDS and renal failure, one each of shock and hemoglobinuria. Fifteen patients had thrombocytopenia. MODS was present in four patients and death was reported in one patient[13]. Another study on 19 patients from Brazil revealed the presence of jaundice in seven patients, ARDS in six patients, MODS in four patients and death was reported in six patients[19]. The study from Papua New Guinea children on 27 patients of severe vivax malaria revealed eight patients of respiratory distress, seven patients of convulsion, six patients of cerebral malaria, five patients of metabolic acidosis/hyper lactatemia, three patients of severe anaemia, two patients of hypoglycemia, and one patient of renal impairment. Jaundice was not present in any patient. Presentation of severe *P. vivax* malaria was similar to severe *P. falciparum* malaria except five times more cases of respiratory distress associated with *P. vivax* monoinfection. They also reported death in one child[17]. It is quite apparent from these studies that India and Brazil have almost similar clinical presentation in the form of increased incidence of jaundice, renal failure and thrombocytopenia along with MODS, whereas in Papua New Guinea the common pre-

sentation is cerebral malaria, metabolic acidosis, respiratory distress and severe anaemia.

There are two studies related to detailed description of cerebral malaria in *P. vivax* monoinfection. The Indian study describes 13 patients of cerebral malaria (2–13 yr) with one death. Eight children had MODS and there was no evidence of neurological sequelae[18]. In an another study from Papua, Indonesia on six adults (16–25 yr), one patient had tremors and myoclonus as a PMNS and there was no death[16]. PMNS has also been reported in other Indian studies in the form of bilateral facial palsy, psychosis and tremors[6,8,28].

There are two retrospective studies from Pakistan in which PCR was done only in few patients and thus the results cannot be compared with other studies. However, the most important complications were cerebral malaria followed by severe thrombocytopenia, severe anaemia and MODS, and death was reported in one patient. The clinical presentation of severe *P. vivax* monoinfection was similar to severe *P. falciparum* monoinfection in both the studies[23,27].

The reports on thrombocytopenia with severe malaria in PCR diagnosed patients of *P. vivax* malaria are very few. In a study by Kochar *et al*[15], on 1064 adult patients, the authors reported severe thrombocytopenia (<20,000) in 39 patients (26 *P. falciparum*, 9 *P. vivax* and 4 mixed infections). They reported that the association of thrombocytopenia was statistically more significant with *P. vivax* monoinfection as compared to *P. falciparum* monoinfection. Six patients (3 *P. vivax*, 2 *P. falciparum* and 1 mixed) had severe epistaxis and required platelet transfusion for management[15]. In a similar study on 676 children, the authors observed that the association of other severe manifestations was significantly more common in children having *P. vivax* monoinfection with severe thrombocytopenia. Severe thrombocytopenia was present in 73 patients and bleeding manifestation was present in 63 patients[21]. Thrombocytopenia was also reported in 15 out of 17 patients in a Brazilian study on severe vivax malaria[13].

Thus, regardless of how this parasite is pernicious, the available data demonstrated that the infections come as a significant burden of morbidity and associated with mortality[39]. In spite of definite evidence of severe *P. vivax* malaria on PCR diagnosed patients from different regions of the world and well supported by autopsy report from India and Brazil[32–33], more detailed prospective, clinico-epidemiological studies are needed to establish whether the increased reports of severe vivax malaria in the past five years are a result of multifocal emergence of virulent strains, previously underestimated severity, or over-reporting[30].

The clinical research on the impact of mixed infection on human health is still controversial, while some workers believe it to be a beneficial situation whereas others consider it to be detrimental[7,11,34–37]. The vast difference in results may be due to large scale incorrect species diagnosis by PBF, attributed to various factors. Previous studies on severe malaria with more number of cases[10–11] have strongly pointed about the limitation of their study with a note of concern that mixed infections might have been largely underestimated and the use of a more sensitive diagnostic method might have had different results.

The magnitude of wrong diagnosis by PBF is evident through a study by Lamph *et al*[16] in which out of 24 patients of PBF diagnosed *P. vivax* monoinfection patients of coma, the PCR was positive for mixed infection in 10 patients. Whereas, in an another study on 180 PBF diagnosed patients of monoinfection [*P. falciparum* 37 (20.5%), *P. vivax* 143 (79.5%) and mixed infection (0)], the cross checking by PCR revealed mixed infection in 82 samples (45.5%), *P. vivax* in 85 (47.2%) and *P. falciparum* in 13 (7.77%)[40]. In a similar study from Papua New Guinea, the authors recorded only 16% concordance with PBF (68% diagnosed as *P. falciparum* and 16% diagnosed as *P. vivax* on microscopy)[17]. Thus, the PCR has a great role in the confirmation of mixed infection and might be an important factor for differences in results of various studies from different parts of the world and thereby making them uncomparable. Accurate identification of the malaria parasite species is important not only for successful treatment, but also to design and develop effective malaria control measures and accurate malaria-epidemiological monitoring. Since, there is only one clinical study which describes difference between clinical presentation, morbidity and mortality pattern of mixed infection *vs P. falciparum* and *P. vivax* monoinfection severe malaria, in which the diagnosis was confirmed by PCR, the present hospital based study using PCR for species diagnosis have specific relevance.

This study revealed that mixed infection severe malaria patients had almost similar clinical and laboratory findings to those of severe *P. falciparum* and *P. vivax* monoinfection malaria (Table 1). However, their subsequent clinical progression to severe illness including MODS and mortality was the highest of the three groups, in spite of the fact that the observations were statistically nonsignificant. Similar observations have also been reported from the only other PCR confirmed study on mixed infection severe malaria in children from Papua New Guinea[17]. Since, all other studies have used PBF for spe-

cies diagnosis, the data cannot be compared because of species diagnosis dilemma.

In the present study, although 47.47% of multiorgan dysfunction was caused by *P. falciparum* malaria, the risk was greatest in patients having mixed infection in comparison to either *P. falciparum* monoinfection or *P. vivax* monoinfection. Potentiality of *P. vivax* malaria for causing multiorgan dysfunction was similar to *P. falciparum* malaria and similar observations were also recorded in previous studies[6,12–14,19]. The mortality was reported in 34 patients (20 *P. falciparum*; 10 *P. vivax*; and 4 mixed) and it was highest in the patients of mixed infection severe malaria. The deaths reported in all the patients in this series had severe manifestations and multiorgan dysfunction (Table 3). While analyzing the individual severe manifestation, it was found that jaundice was the major pernicious syndrome (85.29%) followed by severe anaemia (52.94%), renal failure (47.05%), thrombocytopenia (41.18%) and cerebral malaria (20.59%) and there was no statistical difference in different clinical and parasitological settings.

Exact pathogenesis and organ-specific morbidity caused by *P. vivax* infection remains unrecognized and poorly studied because of a paucity of research in this area. Cytokine production, endothelial activation and pulmonary inflammatory responses are higher during and after *P. vivax* infections than in *P. falciparum* infections of similar parasite biomass. Since, all stages of *P. vivax* are visible in peripheral blood, albeit with partial depletion of mature stages, sequestration is not thought to occur in a significant degree in *P. vivax* malaria or cause end-organ dysfunction in the same manner as *P. falciparum*. Recent *in vitro* data showed that *P. vivax*-infected RBCs do cytoadhere to endothelial cells, *via* ICAM-1 and chondroitin sulphate-A (CSA), with a similar strength but a 10-fold lower frequency than *P. falciparum*-infected RBCs. Another study confirms cytoadherence to the glycosaminoglycans, CSA and hyaluronic acid. The autopsy findings suggest that the significant microvascular obstruction from sequestration of parasitized red cells does not appear to occur in vivax malaria, though it is possible that in some circumstances, more limited cytoadherence to endothelial cells may occur, and may amplify local inflammatory responses in affected organs, such as the lung or placenta. Rosetting, adherence of non-infected to infected RBCs, has been linked to the pathophysiology of severe *P. falciparum* malaria. Rosetting has been described *ex vivo* in vivax malaria; however, its role in *P. vivax* pathophysiology is unknown. The pathophysiology of individual syndrome had been critically reviewed by Anstey *et al*[41]. Recently, in an editorial by Baird[42] had rightly commented that in spite of fact that there is no firm evidence of pathogenesis of severe vivax malaria, there is enough evidence of its reality and its acceptance must not await demonstration of mechanism or cofactors. We have to acknowledge real consequences without regard to their specific genesis.

The strengths of the present study is its prospective hospital based clinical observation study, the use of PCR for diagnosis thereby negating any chances of species misdiagnosis and scientific approach using thorough clinical, bacteriological, radiological and other relevant studies to rule out possibilities of other comorbid condition. Such a rigorous approach has been used in only few studies of severe malaria earlier. Thus, if we analyse the two important epidemiological and clinical issues faced by malariologists since the beginning of this century, our study has some important observations to mention. This study on severe malaria from an area of intense transmission of multiple *Plasmodium* species has identified almost similar phenotype of *P. vivax* and *P. falciparum* monoinfection as well as mixed infection in Bikaner, India. Multiorgan dysfunction was also observed in all three situations. Similar observations have also been reported from other parts of the world in PCR diagnosed patients of severe *P.vivax* malaria and have also been supported by postmortem examination. The second clinical dilemma regarding severe malaria associated with mixed infection was also having similar phenotype presentation with more chances of developing severe illness, more severe clinical course including increased incidence of MODS along with higher rates of mortality and morbidity in comparison to *P. falciparum* and *P. vivax* monoinfection severe malaria.

However, these observations were statistically not significant presumably because of smaller sample size. This important limitation requires a similar multicentric clinico-epidemiological study on a larger population before a general statement can be made firmly. This observation is also supported by the only other PCR based study from PNG. These findings provide a strong impetus for further research on the pathogenic potential of *P. vivax* monoinfection as well as *P. falciparum*/*P. vivax* mixed infections. A further large-scale study involving different countries is required to unearth the underlining pathogenesis and its relation to multidrug resistance. The knowledge of mixed infection is equally important for control measures as well as therapeutic options and future vaccine programme. Thus, every effort to reduce or eliminate malaria burden must also target *P. vivax* and mixed infection along with *P. falciparum* in regions where both these species coexist.

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Conflict of interest

We declare no conflicts of interest.

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