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Clinical, hematological and biochemical profile of malaria cases

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Abstract
Introduction: Mortality has risen in recent years, probably due to increasing resistance to antimalarial medicines. Falciparum malaria remains a major cause of mortality[6]. Thus Malaria remains today as it has been for centuries, a heavy burden in Tropical communities, a threat to non-endemic countries and a danger to travelers.
Methods: All patients who presented to Department of Medicine and proved to be malarial parasite positive through peripheral blood smear or quantitative buffy coat method were included in the study. Performa was prepared which included detailed clinical history, clinical findings and laboratory data, which were analyzed as shown in the proforma.
Results: Fever was reported in all patients, in P.falciparum 13 patients (100%) and in P.vivax 20 patients (100%). Chills and rigors were observed in 11 patients (84.61) of P.falciparum and in 17 patients (85%) of P.vivax. Sweating was the next common symptom. It was present in 8 patients (61.53%) of p.falciparum and in 18 patients (90%) of P.vivax. Headache was present in 8 patients (61.53%) of P.falciparum and in 16 patients (800%) of P. vivax.
Conclusion: Compliations like malarial hepatitis, cerebral malaria, acute renal failure, acute respiratory distress syndrome, algid malaria and hypoglycemia were commonly found with P.falciparum

Keywords: Malaria, P. Vivax, complications

1. Introduction
Malaria is a protozoan disease transmitted by the bite of infected anopheles mosquitoes[1]. Malaria continues to be a major health problem in many parts of the world, with over 2400 million people in some 100 countries at risk of infection [2].
In India malaria situation till 1950's was very grave. With over 75 million cases occurring with 0.8 million deaths. There was near elimination of malaria in 1960's through the extensive use of insecticides and antimalarials. But there was resurgence of malaria noted in mid 1970's [3]. Focal outbreaks due to malaria are of frequent occurrence and morbidity, mortality associated with the disease is alarming [4].
Mortality has risen in recent years, probably due to increasing resistance to antimalarial medicines [5]. Falciparum malaria remains a major cause of mortality [6]. Thus Malaria remains today as it has been for centuries, a heavy burden in Tropical communities, a threat to non-endemic countries and a danger to travelers [1].
Hence, the present study was carried out with a view to obtain the data regarding clinical features haematological and biochemical changes.

2. Methodology
All patients who presented to Department of Medicine and proved to be malarial parasite positive through peripheral blood smear or quantitative buffy coat method were included in the study. Performa was prepared which included detailed clinical history, clinical findings and laboratory data, which were analyzed as shown in the proforma.

2.1 Inclusion Criteria
All patients who presented with fever, and all those who were positive for malarial parasite by peripheral blood smear and/or quantitative buffy coat with no previous treatment were included in the study. Patients were considered to havecerebral malaria, if found to have unarousable coma (glass gow coma scale ≤ 9/14), lasting for at least 30 minutes, after ruling out all other causes of encephalitis. ARF was said to be present if urine output was ≤ 400ml/day with s.creatinine > 3mg /dl. Hypoglycemia was considered if patient had RBS < 40mg/dl. ARDS was considered if patient had bilateral lung infiltrations and P_aO_2 /F_iO_2 ≤ 200mm. Hg. and clinical evidence of elevated left atrial pressure. Algid Malaria was diagnosed if patient had systolic blood pressure < 80 mm Hg with cold clammy skin. Malaria hepatitis was considered to be present if S. Bilirubin > 3mg/dl.

2.2 Exclusion Criteria
All patients with presumptive diagnosis of malaria with no evidence of parasite either on peripheral blood smear and or quantitative buffy coat method. Patient were screened for various systemic manifestations and systemic disturbances, were assessed based on renal function test; liver function test, chest X Ray, Haemogram as and when required.

2.3 Thin Blood Film
Preparation: A drop of blood approximately 3mm in diameter is placed on a clean, grease and moisture free slide 1/3 of the way from the labeled end. A second slide held at 45^0 is used as a spreads to distribute the blood smoothly and rapidly. The blood film is thoroughly air dried.

Six drops of Leishman's stain is placed on the blood smear and it is spread over the film, which acts as initial fixative.
The stain is allowed to fix the smear for approximately three to five minutes and then add 12 to 15 drops of buffer, P^H 7.2 and mix on slide.
The smear is allowed to stain for 7-100 min, then wash with buffer at P^H 7.2 The slide is air dried in a vertical position.
Optimal morphology of malaria parasite is obtained in the lower 1/3 of the smear where red blood cells are just overlapping.
The thin blood film is examined under oil immersion microscopy, with X-100 objective.

2.4 Thick Blood Film

Preparation: Approximately 5ml of blood placed on a clean slide with an applicator stick and is quickly distributed to make an even thick film about $1cm^2$. The thick film is air dried [without heating the slide] in a horizontal position. Slide is dehemoglobinised by putting in distilled water for 5-100 and again air dried.
Leishman stain is applied to the unfixed thick smear and stained for 10minutes, washed in tap water or buffered water, P^H 7.2. The film is then air sried in a rack.

2.5 Examination

Macroscopically the thick film is light blue and semi-transparent. It is examined with a X 100 oil immersion objective.
The thick film will reveal white blood cells and platelets but no RBD's at they are lysed during the staining process. It is more difficult to differentiate the species of malaria as the red cells characteristics [eg. Stippling] are usually absent. Therefore for final species identification thin films are used. QBC method [Beckton Dickinson]

3. Method

1. The QBC capillary tube is filled with blood.
2. The tube is rolled back and forth for 5sec to mix the blood with the contents of the tube.
3. Using forceps, a float is inserted in the capillary tube and then the tube is capped.
4. The tube is centrifuged for 5min at 2,500 r.p.m.
5. The tube is removed from the centrifuge, placed in the para viewer and viewed under UV illumination using oil immersion. Under UV illumination the acridine orange staining parasite DNA fluoresces. The nuclei smaller and more compact than nuclei of leucocytes and the cytoplasm appears orange red compared to the bright green nucleus of the lecocytes. Presence of malarial pigment [brown colour] is also detected here.

4. Results

Totally 35 cases of Malaria admitted to Basappa Memorial Hospital Mysore, who met the inclusion criteria were studied.

Table 1

PBS	QBC	Total Case	Percentage
Positive	Positive	25	71.42
Negative	Positive	5	22.85
Positive	Negative	2	5.71
Chi-square=24.40; P<.000 (S)			
Total QBC positive	33	94.28	
Total PBS positive	27	77.14	

The above table shows total number of malaria positive cases by QBC method and by PBS method. Totally 33 cases were found to be positive by QBC method and 27 positive cases by PBS method, out of 35 cases studied.
Significantly higher number of cases (71.42%) were observed in both positivity for PBS and QBS. This was followed by –ve and +ve of PBS and QBC (22.85%), and least were found in +ve and –ve for PBS and QBC together (5.71%). Further chi-square test revealed a significant difference among these frequencies.

Table 2

Species	No of cases	PERCENTAGE
Pv	20	57.14
Pf	13	37.14
Mixed (Pf and Pv)	2	5.72

Out of 35 cases, 20 (57.14) were Plasmodium Vivax, 13 (37.14%) were Plasmodium faliciparum and 2 (5.72%) were mixed (Pf and Pv).

Table 3: Age and Sex Distribution

Age (in years)	Male	Female	Total	Percentage
< 20	5	2	7	20
21-3	8	-	8	22.85
31-40	8	3	11	31.42
41-50	3	1	4	11.42
51-60	2	1	3	8.57
> 61	1	1	2	5.71
Total	27	8	35	
Percentage	77.15	22.85	100.0	

Mle: Female ration = 3.38:1
Mean Age =33.23 years

The above table shows distribution of age and sex. Males were 27 and females 8. Maximum number of cases occurred in the age group 31-40 years (11 cases).

Table 4: Month Wise Distribution Chart

Years	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
2004	-	-	1	-	5	3	-	3	1	1	-	4
2005	-	1	2	3	-	1	-	-	1	1	-	1
2006	2	-	1	1	3	-	-	-	-	-	-	-
Total	2	1	4	4	8	4	0	3	2	2	0	5

Above table shows month wise distribution of cases in the study period. Maximum number of cases occurred in May, June and August in 2004, in April in 2005 and May and January months in 2006. Thus there is no consistency in the incidence of malaria during any month or season.

In the next following tables, mixed species are not taken in to consideration, for the purpose of comparison between P.falciparum and P. Vivax species.

Table 5: Clinical Symptoms

Symptoms	Species	Male	Female	Percentage	
		Number	Number	Number	Percentage
Fever	Pf	7	6	13	100
	Pv	18	2	20	100
Chills/rigors	Pf	7	4	11	84.61
	Pv	16	1	17	85
Sweating	Pf	7	1	8	61.53
	Pv	18	0	18	90
Headache	Pf	6	2	8	61.53
	Pv	15	1	16	80
Body ache	Pf	2	1	3	23.07
	Pv	10	1	11	55
Vomiting	Pf	4	3	7	53.84
	Pv	6	0	6	30
Jaundice	Pf	2	0	2	15.38
	Pv	2	0	2	10
Diarrhoea	Pf	2	0	2	15.38
	Pv	1	0	1	5
Anorexia	Pf	2	0	2	15.38
	Pv	3	0	3	15
Pain Abdomen	Pf	0	0	0	0
	Pv	2	0	2	0
Altered Sensorium	Pf	1	0	1	7.69
	Pv	1	0	1	5
Joint pain	Pf	0	0	0	0
	Pv	2	0	2	10
Loss of consciousness	Pf	0	1	1	7.69
	Pv	0	0	0	0
Cough/ Breathlessness	Pf	0	0	0	0
	Pv	1	0	1	5
Restlessness	Pf	1	0	1	7.69
	Pv	0	0	0	0

Above table show various clinical symptoms.

Fever was reported in all patients, in P.falciparum 13 patients (100%) and in P.vivax 20 patients (100%). Chills and rigors were observed in 11 patients (84.61) of P.falciparum and in 17 patients (85%) of P.vivax. Sweating was the next common symptom. It was present in 8 patients (61.53%) of p.falciparum and in 18 patients (90%) of P.vivax. Headache was present in 8 patients (61.53%) of P.falciparum and in 16 patients (800%) of P.vivax.

Vomiting was present in 7 patients (53.84%) of P.falciparum and in 6 patients (30%) of P.vivax. Jaundice was seen in 2 patients (15.38%) of P.falciparum and in 2 patients (10%) of P.vivax.

Diarrhoea was present in 2 patients (15.38%) of P. falciparum and 1 patient of (5%) of P. Vivax. Anorexia was present in 2 (15.38%) patients of p. falciparum and 3 patients (15%) of P.vivax. Pain abdomen was found in 2 (10%) patients of P.vivax. one patients (7.69%) of P. falciparum and one patient (5%) of P.Vivax had altered sensorium 2 patients (10%) of P.vivax had joint pain. One patient (7.69%) of p.falciparum had loss of consciousness. Cough/Breathlessness was seen in 1 patient (5%) of P.Vivax. Restlessness in 1 patient (7.69%) of p.falciparum.

Table 6: Clinical Signs

Signs	Species	Male	Female	Percentage	
		Number	Number	Number	Percentage
Pallor	Pf	3	3	6	46.15
	Pv	4	0	4	20
Icterus	Pf	1	0	1	7.69
	Pv	1	0	1	5
Herpes liabialis	Pf	0	1	1	7.69
	Pv	0	0	0	0
Spleen	Pf	4	0	4	30.76
	Pv	7	0	7	35.0
Liver	Pf	1	6	7	53.84
	Pv	1	2	3	15
Meningeal signs	Pf	1	1	2	15.38
	Pv	0	0	0	0
Convulsions	Pf	1	1	2	15.38
	Pv	0	0	0	0

Above table shows clinical signs. Pallor was noticed in 6 patients (46.15%) of p. falcipuram and 4 patients (20%) of P. vivax. Icterus was seen in one patient of P.vivax (5%) and in one patient of P.falciparum (7.69%). Herpes labialis was seen in one patient (7.69%) of p. falciparum.

Spleen was palpable in 4 patients (30.76%) of P. falciparum and 7 patients (35%) of P. Vivax. Liver was enlarged in 7 patients (53.84%) of p.falciparum and in 3 patients (15%) of p.vivax meningeal signs and convulsions were present in 2 patients (15.38%) of p.falciparum.

Table 7: Complications

Complications	Species	Male	Female	Percentage	
		Number	Number	Number	Percentage
Malarial hepatitis	Pf	4	0	4	30.76
	Pv	0	0	0	0
Cerebral malaria	Pf	1	1	2	15.38
	Pv	0	0	0	0
ARF	Pf	1	0	1	7.69
	Pv	0	0	0	0
ARDS	Pf	1	0	1	7.69
	Pv	0	0	0	0
Algid malaria	Pf	1	0	1	7.69
	Pv	0	0	0	0
Hypoglycemia	Pf	1	0	1	7.69
	Pv	0	0	0	0

Above table shows various complications observed. Malarial hepatitis was present in 4 patients (30.76%) of falciparum 2 patients (15.38%) of p.falciparum had cerebral malaria as a complication. Acute renal failure was found in 1 patient (7.69%) of P.falciparum.

Acute Respiratory Distress Syndrome was found in 1 (7.69%) patient, Algid Malaria in 1 patient (7.69%) and Hypoglycemia in one patient (7.69%) of P.falciparum.

Table 8: Haematological Profile

Parameters	Species	Male	Female	Percentage	
		Variations	Variations	Variations	Percentage
Hb	Pf	4	1	2	38.46
	Pv	2	0	2	10
TC	Pf	1	2	3	23.07
	Pv	7	0	7	35
N	Pf	2	1	3	23.07
	Pv	5	0	5	25
L	Pf	1	1	2	15.38
	Pv	1	0	1	5
ESR	Pf	2	0	2	15.38
	Pv	3	1	4	20
Platelets	Pf	2	3	5	38.46
	Pv	8	0	8	40

Table 9: Haematological Profile (Mean)

Parameters	Species	Number	Mean	S.D.
Hb	Pf	13	10.21	3.85
	Pv	20	12.45	1.63.
TC	Pf	13	7338.46	3902.03
	Pv	20	6045.00	3326.20
N	Pf	13	67.0	13.84
	Pv	20	65.1	14.17
L	Pf	13	35.38	11.82
	Pv	20	31.00	9.07
ESR	Pf	13	33.84	45.10
	Pv	20	24.05	19.79
Platelets	Pf	13	152780.0	109146.20
	Pv	20	150114.9	112486.03

Above table shows variations in haematological profile.

Mean haemoglobin in P.falciparum was 10.21±3.85 and 4 patients (30.76%) had variations. In case of P. vivax, mean haemoglobin was 12.45± 1.63 and 2 patients (10%) had variations.

Total Leucocyte count ranged from 2100 cells / mm^3 to 17,000 cells /mm^3. Mean was 7338.46± 3902.03, in p.falciparum and 6045 ± 3326, in P.vivax. 3 patients (23.07%) of p.falciparum and 7 patients (35%) of p.vivax had variations. Mean value of Neutrophil count was 67 ± 13.84, in P. falciparum and 65. ± 14.17, in P.Vivax. 3 patients of P.falciparum and 5 patients of P.vivax had variations. Mean value of lymophyocyte count was 35.38 ± 11.82, in P.falciparum and 31 ± 9.07, in P.vivax. 2 (15.38%) patients in P.falciparum and 1 (5%) patient in P.vivax had variations. In the monocyte and eosinophil count, none had variations.

Mean value of ESR was 33.84 ± 45.10, in P.falciparum and 24.05 ± 19.79, in P.vivax 2 (15.38%) patients of p.falciparum and 4 (20%) patients of P.vivax had variations.

Mean value of thrmobocytes was 1,52780 ± 109146.20, in P.falciparum and 1,50114.9 ± 112486.03, in P.vivax. 5 patients (38.46%) of P.falciparum and 8 patients (40%) of P.Vivax had variations.

Table 10: Biochemical Profile

Parameters	Species	Male	Female	Percentage	
		Variations	Variations	Variations	Percentage
Sodium	Pf	3	1	4	30.76
	Pv	5	0	5	25
Potassium	Pf	1	1	2	15.38
	Pv	2	0	2	10
Chloride	Pf	3	1	4	30.76
	Pv	4	0	4	20
B.Urea	Pf	1	1	2	15.38
	Pv	0	0	0	0
S.Creat.	Pf	1	2	3	23.07
	Pv	0	0	0	0

Table 11: Biochemical Profile (Mean)

Parameters	Species	Number	Mean	S.D.
Sodium	Pf	13	135.38	4.48
	Pv	20	136.40	5.65
Potassium	Pf	13	4.02	0.48
	Pv	20	4.28	0.54
Chloride	Pf	13	102.69	5.20
	Pv	20	100.80	4.68
B.Urea	Pf	13	25.08	20.97
	Pv	20	22.80	6.50
S.Creat.	Pf	13	1.64	0.74
	Pv	20	1.11	0.60

Mean value of Sodium was 135.38 ± 4.48, in P.falciparum and 136.40 ± 5.56, in P.Vivax. 4 (30.76%) patients of P.falciparumand 5 (25%) patients of P.vivax had variations. Mean value of Potassium was 4.02 ± 0.48, in P.falciparum and 4.28 ± 0.54, in P. Vivax, 2 (15.38%) patients of P.falciparum and 2 (10%) patients of P.vivax had variations. Mean value of Chloride was 102.69 ± 5.20, P.falciparum and 100.80 ± 4.68, in P.vivax. 4 (30.76%) patients of P.falciparum and 4 (20%) patients of P.vivax had variations.

Mean value of B.urea was 25.08 ± 20.07, in P.falciparum and 22.80 ± 6.50, in P.vivax, 2 (15.38%) patients of P.falciparum had variations. Means value of S.creatinine was 1.64 ± 0.74 in P.falciparum and 1.11 ± 0.60 in P.vivax. 3 (23.07%) patients of P.falciparum had variations. None of the P.vivax patients had variations in B.urea and S.Creatinine.

Table 12: Outcome

Cases	Improved	Expired
Pf	12	1
Pv	20	0

One patient (7.69%) of P.falciparum expired. All patients of P. Vivax improved.

Discussion

Malaria should be considered as a possibility in all patients presenting with high grade intermittent fever with chills unless proved otherwise, specially when the person is hailing from endemic areas. In this, out of 35 cases of proved malaira 33 (20 cases of P.Vivax and 13 cases of P.falciparum) were studied to find out the different modes of clinical presentation, systemic complications, haematological and biochemical changes in malaria. And 2 cases (mixed species) were excluded from the study for the purpose of comparison of P. falciparum and P. Vivax.

In my study total number of smear positive cases are 27 (77.14%) and QBC positive case are 33 (94.28%). This shows that conventional microscopy is limited by being highly observer dependent and even a carefully examined blood film may be negative for parasites due to previous treatment, highly synchronous infection or sequestration of parasitized RBC's in the internal organ. Similar observation was made by Tanes Singhal *et al.* [7] and Meenal Jain *et al.* [8].

In the present study, P.vivax (57.14%) cases were more than P.falciparum (37.14%). This was similar to the observation made by U.M. Jadhav *et al.* [9] and Laura M. *et al.* In Gaffer *et al.* [10] study P.falciparum cases (90.4%) were more than P.vivax (9.6%). This is because species predominate depending upon the geographic distribution and also drug resistance.

Maximum prevalence was noticed in the age group of 31-40 years, with mean age of 33.23 years. In Gaafer M. Malik *et al.* [10] study, age group affected was below 15 years. And in Richard Idro *et al.* [11] study, bimodal age peak was observed. This is because our area belongs to low endemicity zone and age shifts upwards in lower endemic areas. Also waning of immunity plays a role.

In the present study males out numbered the females. This may be because males are less covered by clothes than females, also because of their outdoor activities and women visit doctors less frequently low socioeconomic areas. These findings correlate with that of U.M. Jadhav *et al.* [9].

In my study there was no correlation with the incidence of malaria and particular season. In Lulu Muhe *et al.* [12]. 30.3% of malaria occurred from April onward. In Gaafer. M. Malik *et al.* [10]. Study there was a peak following rainy season and in summer. This difference is probably due to the variation in the parasite density, distribution and improved vector preventive measures.

In my study fever was found in all patients (100%). It was intermittent in 20 patients (100%) of P.vivax and in 12 patients (92.23%) of P. falciparum and continuous in 1 patient (7.69%) of P.falciparum. widal test was positive and blood culture was negative. This indicates that associated infection in a proved case of malaria should always be ruled out, in any case of atypical presentation of fever. Similar observations were made by Samal K.K. *et al.* [13] All patients had received antipyretics and none of them had hyperpyrexia and did not had classical periodicity of malaria.

Herpes labialis was found in one patient (7.69%) of P. falciparum. This is similar to the study done by Maj. S. R. Mehta *et al.* [14].

Spleen was palpable in 4(30.76%) patients of P. falciparum and in 7 (35%) patients of P.Vivax. In Hazra B R *et al.* [15] study splenomegaly was found in 40% in P. falciparum and 18.8% in P. vivax.

Icterus was present in 1 patient (7.69%) of P. falciparum and 1 (5%) patient of P. Vivax. Hepatitis was found in 4 (30.76%) patients of P.falciparum. Hepatomegaly was found in 7 (53.84%) patients of P.falciparum and 3 (15%) patients of P. Vivax. In Hazra BR *et al.* [15] study icterus was noted in 40% with P.falciparum and 9.09% with P.vivax and Hepatomegaly in 80% of P.falciparum and 63.63% of P.vivax.

Mean value of B.Urea in P.falciparum (25.08 ± 20.97) was more than in P. Vivax (22.80 ± 6.50). Two (15.38%) patients had raised blood urea in P.falciparum and none in P.vivax. The mean value of S. creatinine was more in P.falciparum (1.64 ± 0.74) than in P.Vivax (1.11 ± 0.60). 3 patients had raised S.creatinine in P.falciparum and none in P.vivax. The raised blood urea and S. creatinine were associated with low survival rate and required dialysis in a similar study done by Rubina Naqvi *et al.* [16]. In my study acute renal failure was found in one patient (7.69%) of P. falciparum. None of the patients in P.Vivax had acute renal failure. In several studies acute renal failure has occurred in both species. As in J. Prakash *et al.* [17] study ARF was found in 80.9% of P.falciparum and 11.7% in P. Vivax and in Rubina Naqvi *et al.* [16] study out of 124 ARF patients 121 constituted P.falciparum and only 3 were P.Vivax.

The mean value of sodium was 135.38 ± 4.48 in P.falciparum and 136.40 ± 6.65 in P.vivax four (30.76%) patients patients had hyponatremia in P.falciparum and five (25%) in P.vivax. None of the patients had sodium levels less than 120 mEqui./L. Thus hyponatremia was mild and got corrected after adequate hydration. This was similar to the study done by M.C. English *et al.* [18]. Many studies have shown the role of inappropriate secretion of antidiuretic harmone in hyponatremia, as in the study done by Holst. F.G *et al.* [19].

Mean value of Serum potassium was 4.02 ± 0.48 in P.falciparum and 4.28 ± 0.54 in P.vivax 2 patients (15.38%) of P. falciparum and 2 patients (10%) of P.vivax had mild hypokalemia. Mean value of Chloride was 102.69 ± 520 in P. falciparum and 100.08 ± 4.68 in P.vivax. 4 patients (30.76%) in P.falciparum and 4 patients (20%) in P.vivax had hypochloremia.

In my study, the mean duration of the hospital stay of P.Vivax cases was 4.5 days compared to 7.5 days in P.falciparum. Out of 13 cases of P.falciparum, one patient (7.69%) with multi organ failure expired. Overall mortality in my study was 3.03% and the morbidity and mortality was more in P.falciparum cases compared to P.vivax.

Conclusion

- Malaria is the most common disorder in this country presenting with febrile illness and varied clinical manifestrations, with multisystem involvement.
- QBC method was found more sensitive in diagnosing malaria cases, compared to microscopy method.
- In both P.falciparum and P.vivax the predominant clinical symptoms were fever, chills and rigors, sweating, headache, bodyache and vomiting.
- Altered sensorium, loss of consciousness, restlessness were more common in P.falciparum than P.vivax.
- Pallor, heapatomegaly were common clinical signs in P.falciparum than in P.vivax. meningeal signs and convulsions were confined to P.falciparum. splenomegaly was more common in P.vivax than in P.falciparum

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