

## Biochemical characteristics of Malaria patients with their association with severity of disease

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**Abstract**— Malaria is one of the oldest and most widespread disease which affects more than 2400 million people, over 40% of world's population, in more than 100 countries in tropics from South America to Indian Peninsula. This study was designed to assess the platelet counts, haematocrit & liver enzymes (Alanine transaminase, Aspartate transaminase, Alkaline phosphatase) in patients of Plasmodium vivax & Plasmodium falciparum malaria and its association with the severity and prognosis of disease. In a hospital based observational descriptive study, 100 patients with Plasmodium falciparum and/or vivax positive diagnosed by peripheral blood film examination and/or by MPQBC (Malaria Parasite Quantitative Buffy Coat) method were included and submitted to a complete clinical & laboratory evaluation. Patients who were  $\leq 14$  years and who refused to give consent were excluded. They were divided into Plasmodium vivax and Plasmodium falciparum positive group; evaluated, compared and statistical analysis done. Out of total 100 cases of malaria positive, P. falciparum constituted 66% and P. vivax constituted 34%. The pathophysiological processes causing the haematological changes in malaria are complex and multiple. Thrombocytopenia presents with bleeding manifestations and it increases the severity of disease with poor prognosis.

**Keywords:** P. Vivax, P. Falciparum, Platelet, Haematocrit, Liver enzymes.

### I. INTRODUCTION

Malaria is one of the oldest and most widespread diseases in the world which affects more than 2400 million people, over 40% of world's population, in more than 100 countries in tropics from South America to Indian Peninsula.<sup>1, 2</sup> Malaria ranks third among the major infectious disease in causing death after pneumococcal acute respiratory infection and tuberculosis. The term 'malaria' has been derived from Italian description meaning - Bad Air.<sup>2</sup>

Malaria in man is caused by four distinct species of the malaria parasite - P. vivax, P. falciparum, P. malariae and P. ovale. P. vivax has the widest geographic distribution. In India, about 70% of the infections are reported to be due to P. vivax; 25-30% due to P. falciparum and 4-8% due to mixed infection.<sup>3</sup>

The classic presentation of malaria consists of paroxysms of fever alternating with periods of fatigue. Symptoms associated with febrile paroxysms include high grade fever, chills, rigor, sweat, headache, myalgia, back pain, abdominal pain, nausea, vomiting, diarrhea, pallor and jaundice. Paroxysms coincide with rupture of RBC laden with Schizonts that occur every 48 hour with P. vivax resulting in alternate day fever spikes. Periodicity is less apparent with P. falciparum and mixed infections.<sup>4, 5</sup> Plasmodium falciparum is the most severe form of malaria and is associated with more intense

parasitaemia.<sup>4</sup>

Clinical assessment of severity depends on bedside observation of variables such as blood pressure fever and mental alertness. These can be supplemented by routine laboratory measurement. The Degree of Parasitaemia is correlated with severity of disease.<sup>6</sup>

Over the last decades, a number of observations have suggested that platelets may play a role in the patho-physiology of severe Malaria. In severe Malaria thrombocytopenia (Platelets count <150x10<sup>9</sup>/L) strongly correlates with high levels of interleukins (IL-10) these data suggest why thrombocytopenia has a complex relationship with severe disease.<sup>7</sup>

Haematocrit is a key factor in the interaction between Malaria infected RBC and microvascular endothelial cells. Scientists in Canada explained - Volume fraction of erythrocytes (Haematocrit) and their physical properties such as deformability are important properties of blood that affect cell recruitment to the vascular wall.<sup>8</sup>

Mild haemolytic jaundice is common in Malaria. Severe jaundice is associated with *P. Falciparum* infections which are more common among adults than children and results from hemolysis, hepatocyte injury and cholestasis; when accompanied by other vital organ dysfunctions (often renal impairment), it carries a poor prognosis.<sup>9</sup>

This study was conducted to find out the platelet counts, haematocrit & liver enzymes (SGOT, SGPT, Alkaline phosphatase) in patients of *Plasmodium vivax* & *Plasmodium falciparum* malaria and its association with the severity and prognosis of the disease.

## II. METHODOLOGY

A hospital based observational descriptive type of study was conducted at SMS Medial College and Hospital, Jaipur (Rajasthan). Subjects, who found to be malaria parasite positive by PBF and/or MPQBC testing, were enrolled in the study. Cases with age group  $\leq 14$  years and who refused to give consent were excluded. Finally 100 eligible Malaria positive subjects were enrolled in this study. All patients gave informed consent and the study protocol was approved by the ethics committee of the hospital.

Out of total 100 cases of malaria positive by PBF/MPQBC method, *P. Falciparum* constituted 66% (n=66) and *P. Vivax* constituted 34% (n=34). The baseline physical characteristics and investigations were compared in these two groups using statistical tests of significance.

Detailed clinical history and thorough physical examination was carried out and blood samples were collected after at least twelve hours overnight fasting for complete blood counts, haematocrit, blood glucose, blood urea, serum creatinine, liver functions (total bilirubin, direct and indirect bilirubin, alanine transaminase (SGOT), aspartate transaminase (SGPT), alkaline phosphatase (ALP), gamma glutamyl transferase, serum total protein, serum albumin, serum globulin) in all participants. All patients were evaluated for various presenting complications followed till day 5 or discharge or death. All were treated with injectable chloroquine, artesunate or quinine until patients were able to take the drug orally.

### 2.1 Preparation of Blood Films

Blood films used for detection of malarial parasites are: (a) Thin film (b) Thick film. (c) Thin and thick

film. These films were stained with either Giemsa stain or JSB Stain (Jaswant Singh, Bhattacharya, 1944; rapid Romanowsky method of staining of malaria parasites). A rough estimate of parasite concentration was provided by showing average number of parasites observed per oil immersion field (01 F) or thick film-as follows:

+	1-10 parasites per 100 fields of thick film
++	11-100 parasites per 100 fields of thick film
+++	1 -10 parasites per fields of thick film
++++	> 10 parasites per fields of thick film

A thick smear was diagnosed as negative whereas no asexual forms (of PR) were observed in 200 fields according to 1998 WHO protocol.

## 2.2 Quantitative Buffy Coat (QBC) method<sup>2</sup>

QBC tube method is a new direct rapid diagnostic technique for malaria. QBC procedure is a registered trademark of Becton Dickinson and Company (Franklin Lakes NJ; USA). (Method employed was as per Instructions given by the company manufacturing the kit).

## 2.3 Test Principle

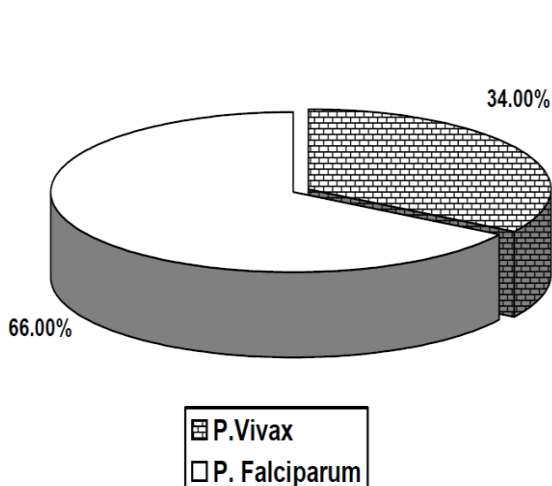
The QBC malaria detection test is based upon the density gradient layering of red cells within microhaematocrit tube. Red blood cells infected with malarial parasites appear to be less dense than uninfected red blood cell and on centrifugation, concentrate primarily in a zone at the interface between granulocyte and red blood cell layer.

Data was analyzed by SPSS version 16 (trial version) using student chi square test and 'p' value <0.05 will be considered as significant.

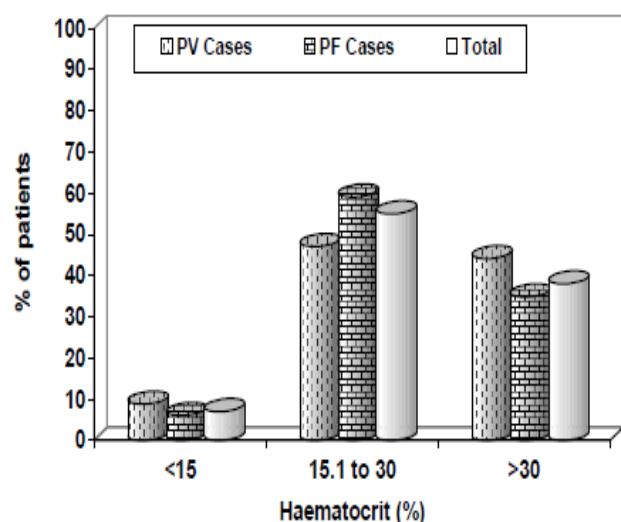
## III. RESULT

Out of total 100 cases of malaria positive by PBF/MPQBC method, P. Falciparum constituted about 66% and P. Vivax constituted about 34% of total patients. (Figure 1)

**Figure 1**  
Type of Malaria wise distribution



**Figure 2**  
Haematocrit percentage wise distribution



Out of 34 P. Vivax positive cases, 32.35%, 41.17%, 26.48% patients were from age <25 yrs, 25 to 50yrs, >50 yrs respectively. Out of 66 P. Falciparum positive cases, 31.82%, 53.03%, 15.15% patients were from Age <25 yrs, 25 to 50yrs, >50 yrs respectively. Most common age group affected was between 25 to 50 yrs.

In P. Vivax positive cases, fever (100%) followed by headache (76.47%), vomiting (64.70%), pallor (47.05%) and jaundice (38.23%) was the most common presentation. In P. Falciparum positive cases, fever (100%), followed by vomiting (75.76%), pallor (74.24%), jaundice (65.15%) and headache (59.09%) was the most common presentation. (Table 1)

**Table 1**

**Comparison of signs and symptoms**

S. No.	Symptoms./Signs	PV Cases		PF Cases	
		No.	%	No.	%
1	Fever	34	100	66	100
2	Pain abdomen	1	2.94	1	1.51
3	Vomiting	22	64.7	50	75.76
4	Loose motion	7	20.58	28	42.42
5	Headache/Myalgia	26	76.47	39	59.09
6	Cough	4	11.76	4	6.06
7	Altered Sensorium	3	8.82	21	31.81
8	Decrease Urine Output	1	2.94	28	42.42
9	Epistaxis/Gum bleeding	7	20.58	31	46.97
10	Rash	4	11.76	17	25.76
11	Pallor	16	47.05	49	74.24
12	Jaundice	13	38.23	43	65.15
13	Splenomegaly	27	79.41	58	87.87
14	Hepatomegaly	10	29.41	29	43.93

Episodes of epistaxis/Gum bleeding/skin Rashes was found in 20.58% of P. Vivax positive and 46.97% of P. Falciparum positive patients.

In 47.06% of P. Vivax positives and 62.13% of P. Falciparum positives had haemoglobin level less than 10gm%. Likewise, 55.85% P. Vivax positive and 65.15% P. Falciparum positive patients had haematocrit level less than 30% (P>0.05). Severity of anaemia was also of greater degree in patients of P. Falciparum (Table 2; Figure 2).

**Table 2**

**Comparison of Haematocrit level in malaria cases**

S. No.	Haematocrit (%)	PV Cases		PF Cases	
		No.	%	No.	%
1	<15	3	8.82	4	6.06
2	15.1 to 30	16	47.06	39	59.09
3	>30	15	44.12	23	34.85
	<b>Total</b>	<b>34</b>	<b>100</b>	<b>66</b>	<b>100</b>

P>0.05 (NS)

Out of 34 P. Vivax positive cases, 35.29% were having leucopenia and 11.77% were having leucocytosis. Out of 66 P. Falciparum positive cases, 24.24% were having leucopenia and 10.61% were having leucocytosis. Leucopenia and leucocytosis is more common with P. Vivax malaria ( $P>0.05$ ).

Out of 34 P. Vivax cases, 73.52% patients were having platelet count  $<1, 50, 000/mm^3$ . The minimum platelet count observed was  $8000/mm^3$  and was associated with bleeding manifestations. Out of 66 P. Falciparum positive cases, 89.38% patients were having platelet count  $<1,50,000/mm^3$ . The minimum platelet count observed was  $4000/mm^3$  and was associated with bleeding manifestations ( $P>0.05$ ) (Table 3).

**Table 3**

**Comparison of Platelet count in malaria cases**

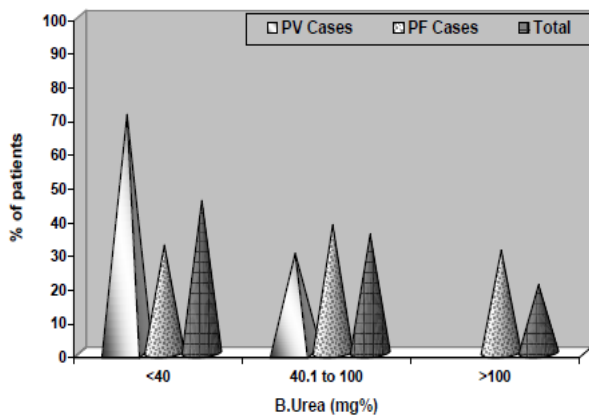
S. No.	Platelet count/ $mm^3$	PV Cases		PF Cases	
		No.	%	No.	%
1	$<20,000$	5	14.70	19	28.78
2	20 to 50,000	10	29.41	24	36.36
3	50,000 to 1.5 lacs	10	29.41	16	24.24
4	$>1.5$ lacs	9	26.48	7	10.62
	<b>Total</b>	<b>34</b>	<b>100</b>	<b>66</b>	<b>100</b>

$P>0.05$  (NS)

Out of 34 P. Vivax cases, 20.59% patients were having S. Creatinine level  $>1mg\%$  and 29.41% patients were having blood urea  $>40mg\%$ . Out of 66 P Falciparum positive patients, 57.58% patients were having S. Creatinine level  $>1mg\%$  and 68.18% patients were having blood urea  $>40mg\%$ . Renal function was more dearranged with P. Falciparum malaria ( $P<0.05$ ). (Figure 3 & 4)

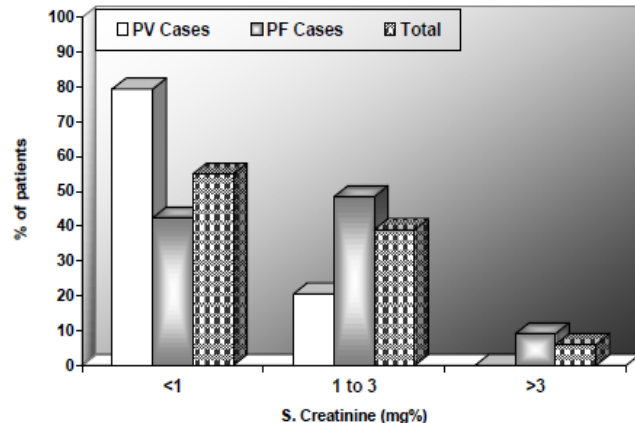
**Figure 3**

**Blood Urea in P. vivax and P. falcifarum**



**Figure 4**

**S. Creatitine in P. vivax and P. falcifarum**



Out of 34 P. Vivax positive cases, 55.88% patients were having SGOT level  $>40U/L$ , 52.95% patients were having SGPT level  $>40 U/L$ , 64.70% patients were having Alkaline Phosphatase  $>125 IU/L$ . Out of 66 P. Falciparum positive cases, 84.85% patients were having SGOT level  $>40U/L$ , 74.24% patients were having SGPT level  $>40 U/L$ , 77.27% patients were having Alkaline Phosphatase  $>125 IU/L$ . Liver Dysfunction was present in both P. Falciparum and P. Vivax infection but significantly more common with P. Falciparum malaria ( $P<0.05$  for SGPT) (Table 6).

**Table 6****Comparison of SGPT levels in malaria cases**

S. No.	SGPT U/L	PV Cases		PF Cases	
		No.	%	No.	%
1	<40	16	47.05	17	25.76
2	40-200	17	50.00	49	74.24
3	>200	1	2.95	0	00.00
	<b>Total</b>	<b>34</b>	<b>100</b>	<b>66</b>	<b>100</b>

P&lt;0.05 (S), SGOT – Serum glutamic pyruvic transaminase

Majority of complications in malaria in this study were found to be caused by *P. Falciparum*. ARF and cerebral malaria and were found significantly more in *P. Falciparum* malaria (P<0.001) than in *P. Vivax* positives. (Table 5)

**Table 5****Comparison of Complications in cases of malaria**

S. No.	Symptoms./Signs	P. Falciparum		P. Vivax	
		No.	%	No.	%
1	Cerebral Malaria	21	31.81	3	8.82
2	Acute Renal Failure	28	42.42	1	2.94
3	Congestive Heart Failure	00	00.00	0	00.00
4	Acute Respiratory Distress Syndrome	02	3.03	0	00.00
5	Hypotension	04	6.06	2	5.88
6	Electrolyte Imbalance (Hyponatremia)	04	6.06	2	5.88

P&lt;0.001 (HS)

#### IV. DISCUSSION

There is continuing challenge of malaria infection due to Resurgence, Drug resistance, varying clinical presentations and various complications.

Fever was the hall mark of disease and present in all (100%) the cases of malaria studied in both the groups. Fever of different grades and patterns with chills and rigor have been reported in most of the studies. Hazra et al observed that fever was present in all cases of *P. Vivax* and *P. Falciparum* malaria.<sup>10</sup> Gautam et al reported chills and rigors in 75% cases with malaria due to stimulation of autonomic system.<sup>11</sup> The fever was caused by release of pyrogen by malaria parasites.<sup>12</sup>

Gastro-intestinal symptoms such as vomiting and loose motion were the chief presenting complaints; vomiting was present in 75.76%, 64.70% cases and loose motion was present in 42.42%, 20.58% cases of *P. Falciparum* and *P. Vivax* positive case respectively. Gautam et al observed diarrhea in 35% and vomiting in 37% of malaria cases.<sup>11</sup> Lutalo et al found diarrhea and vomiting in 47.7% cases of malaria.<sup>13</sup> While recently Hazra et al reported Nausea/vomiting in 60% and 45.5% cases of *P.*

Falciparum and *P. Vivax* respectively and abdominal pain/diarrhea in 10% and 9.09% in *P. Falciparum* and *P. Vivax* respectively.<sup>10</sup> GIT involvement in malaria is due to vascular congestion leading to various manifestations like vomiting, diarrhea, pain abdomen, gastrointestinal bleeding etc.

In present study, 29.41% of *P. Vivax* cases were having blood urea >40mg% and 20.59% were having serum creatinine more than 1 mg%. Whereas 68.18% of *P. Falciparum* cases were having blood urea >40 mg and 57.58% were having serum creatinine >1 mg%. The incidents of renal involvement in malaria, as reported in many studies, varied from 5 to 25%. The renal involvement is more common in patients with *P. Falciparum* malaria than in the *P. Vivax* group of patients<sup>11</sup>. Involvement of kidneys in malaria is multifactorial and may result from release of vasoactive substances causing hypoperfusion, glomerular involvement and/or intravascular hemolysis.<sup>14</sup>

In the present study, 8.82% of *P. Vivax* cases and 31.81% of *P. Falciparum* cases were having altered sensorium. Clinically any degree of impaired consciousness with fever, CNS dysfunction and demonstration of parasite in PBF is considered as cerebral malaria. CNS involvement in cerebral malaria is mainly due to capillary sludging, microcirculatory blockage, changes in blood flow, anaerobic glycolysis and vasculomyelopathy, hypergenetic-antigen antibody reaction.<sup>14</sup> Ahmed et al studied 30 cases of cerebral malaria (26 cases- *P. Falciparum* and 4 case – *P. Vivax*) and found altered sensorium in all cases, 23 cases had convulsions out of which 19 were due to *P. Falciparum* and rest 4 were due to *P. Vivax*.<sup>15</sup>

Anemia is the most frequent hematological abnormality in malaria. It results from destruction of infected RBC during each cycle of schizogony. In addition autoimmune destruction and sequestration of RBCs in reticuloendothelial system, dyserythropoiesis, increased fragility of RBC consequent to electrolyte change, bone marrow depression by malarial toxins and maturation arrest of RBC have also been attributed.<sup>16</sup> Anemia is observed with varying severity in 70 to 80% patients suffering from malaria.<sup>17</sup> In present study, severe anaemia (Hb <5gm %) was observed in 8.82% of *P. vivax* positive cases and 6.06% of *P. Falciparum* positive cases. While other studies have reported moderately higher incidence of severe anaemia ranging from 13%-38%.<sup>14, 17</sup>

Thrombocytopenia was in 89.38% of cases of *P. Falciparum* and 73.52% of cases of *P. Vivax* but more severe decline in platelet count (<20000) was observed in cases of *P. Falciparum*, presented with bleeding manifestations. Thrombocytopenia is also a well known haematological abnormality reported in various studies. Thrombocytopenia is mainly due to splenic pooling of platelets, decrease life span and immune destruction of platelets.<sup>18</sup>

In this study, 50% of *P. Vivax* cases were having SGOT level between 40-200 U/L and 5.88% were having SGOT level >200U/L. Whereas 81.82% of *P. Falciparum* cases were having SGOT level between 40-200 U/L and 3.03% were having SGOT level >200 U/L. Among *P. Vivax* positive cases, 52.95% were having SGPT level >40 while 74.24% of *P. Falciparum* positives were having SGPT level >40. This is mainly due to damage to hepatocyte, microangiopathic hemolysis, sinusoidal congestion and kupffer cell hyperplasia.<sup>19</sup> In a study of complicated *Falciparum* malaria, all patients (100%) had hepatic dysfunction including Jaundice (100%), Ascites (31.25%), hepatomegaly (75%), raised bilirubin and transaminase levels.<sup>92</sup> Jaundice is remarkably common feature of *P. Falciparum* (40%) cases as opposed to *P. Vivax* (9%) cases.<sup>20</sup>

Patwari A et al (1979) observed raised level of SGOT, SGPT and serum alkaline phosphatase in 68, 39 and 46% respectively in 80 children with *P. Vivax* malaria.<sup>21</sup> L.S. et al (1989) serum transaminases were high in 21.8% of patients and S. alkaline phosphatase in 9.37% patients.<sup>22</sup>

## V. CONCLUSION

This present study concludes that the patho-physiological processes causing the haematological changes in malaria are complex and multiple. Thrombocytopenia presents with bleeding manifestations and it increases the severity of disease with poor prognosis. Hepatitis is also found with Malaria. The So malaria case should be treated at earliest to prevent various complications associated with this.

## CONFLICT OF INTEREST

None declared till now.

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