



Evaluation of Hematological and Biochemical parameters in Malaria positive patients- A hospital based cross-sectional observational study

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ABSTRACT

Introduction: Malaria remains a major public health problem in tropical countries, especially in India leading to anemia, thrombocytopenia, leucopenia, hyperbilirubinemia and if untreated; it may damage kidney and liver. It is associated with significant hematological and biochemical alterations that reflect disease severity and prognosis.

Aims and objectives: To evaluate hematological and biochemical parameters in patients diagnosed with malaria.

Methods: This was a hospital-based prospective observational study conducted in the Department of Pathology and Medicine over a period of 6 months from June 2025 to December 2025 in over 591 Malaria positive patients in the Departments of Pathology and Medicine, Bharatratna Dr. Babasaheb Ambedkar Municipal General Hospital, Mumbai. Hematological parameters were analysed using automated hematology analyzers and biochemical parameters were assessed using fully automated biochemistry analyzer.

Results: Anemia, thrombocytopenia and leucopenia were the most common hematological findings. Among the biochemical parameters; hyperbilirubinemia was the most common abnormal biochemical finding with blood urea, serum creatinine, Serum Glutamic- Oxaloacetic Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), alkaline phosphatase and serum protein also showing alterations.

Conclusion: Evaluation of Hematological and biochemical parameters in malaria can serve as an important diagnostic and prognostic indicators to prevent and to monitor liver, kidney and multi-organ dysfunction.

Key-words: Malaria, Hematology, Biochemical parameters, Thrombocytopenia

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INTRODUCTION

Malaria is a major health problem in developing countries particularly in tropical and subtropical regions. It is a mosquito-borne parasitic disease caused by protozoa of the genus *Malaria*, transmitted through the bite of infected female *Anopheles* mosquito. Five species of intracellular protozoa of the genus *Plasmodium* cause malaria in humans. They include *Plasmodium falciparum*, *Plasmodium vivax* (*P. vivax*), *Plasmodium ovale* (*P. ovale*) and *Plasmodium malariae* (*P. malariae*), *Plasmodium knowlesi* (*P. knowlesi*) among which *P. falciparum* and *P. vivax* are the most prevalent in the Indian subcontinent. It is known that *P. falciparum* is associated with severe and life-threatening complications. Malaria control in India is primarily implemented through the National Centre for Vector Borne Diseases Control under the National Malaria Elimination Programme (NMEP), which focuses on early case detection, prompt diagnosis using peripheral blood smear microscopy and rapid diagnostic tests (RDTs), complete treatment with artemisinin-based combination therapy (ACT), vector control, surveillance, and community awareness. In our hospital, these strategies are effectively utilized by performing rapid malaria antigen testing alongside confirmatory peripheral smear examination, supported by routine hematological and biochemical investigations. This integrated laboratory approach facilitates early diagnosis, timely initiation of treatment, identification of severe malaria, and improved patient outcome. Malaria is characterized by a wide spectrum of clinical manifestations ranging from uncomplicated febrile illness to severe complications such as cerebral malaria, acute renal failure, metabolic acidosis and multi-organ dysfunction.^[1] These complications are often associated with significant alterations in hematological and biochemical parameters, which can aid in early diagnosis, assessment of disease severity, and monitoring of treatment response.

Hematological abnormalities are the most common findings in malaria patients with anemia, thrombocytopenia, leucopenia being

the common hematological abnormalities observed. Thrombocytopenia is a common finding which is a useful indicator of malaria. Anemia is another important finding in malaria patients caused due to hemolysis of parasitized red blood cells, increased splenic clearance and ineffective erythropoiesis. Leukocyte count may vary with leucopenia or leukocytosis observed depending on disease severity and host immune response. These changes in hematological parameters can provide valuable diagnostic and prognostic information.^[2,3,4]

In addition to hematological changes, malaria also shows significant biochemical alterations. Liver function test abnormalities including elevated serum bilirubin, SGPT, SGOT and alkaline phosphatase are observed due to hemolysis and hepatic involvement. Renal function impairment may manifest as elevated serum creatinine and urea levels, particularly in severe patients. These parameters serve as important markers for assessing disease severity, guiding therapeutic decisions and predicting patient outcomes. Given the high burden of malaria and its varied clinical presentations, there is a need for comprehensive evaluation of laboratory parameters associated with the disease manifestation. Therefore, this study aims to evaluate the hematological and biochemical parameters in patients of febrile illness diagnosed with malaria.

Methods

Study Design

This is hospital-based observational cross-sectional study for duration of 6 months conducted in the Department of Pathology and Medicine, Bharatratna Dr. Babasaheb Ambedkar Municipal General Hospital, Mumbai.

Study population

This study includes 591 patients who were tested positive for malaria on peripheral blood smears and rapid malaria antigen test. Patients with known hematological disorders, chronic liver or renal disease and patients on chemotherapy were excluded from the study.

Methodology

This study is carried out in Department of Pathology, Bharatratna Dr. Babasaheb Ambedkar Municipal General Hospital Mumbai over a period of 6 months. Venous blood samples were collected under aseptic conditions in EDTA vacutainer tube for hematology analysis and serum gel vacutainer tubes for biochemical analysis. The blood for hematological parameters is analyzed using Erma PCE 525 five part hematology analyzer. The blood for biochemical parameters is analyzed using biochemistry analyzer Erba XL 640 a fully automated biochemistry analyzer. Diagnosis of malaria was confirmed by peripheral smear and Rapid malaria antigen tests. In *Plasmodium falciparum*, ring forms and crescent-shaped gametocytes are typically observed in peripheral blood, whereas *Plasmodium vivax* commonly shows ring forms, mature trophozoites, schizonts and round gametocytes. Rapid malaria antigen test is based on principle of antigen antibody reaction that detects specific proteins like HRP2 or pLDH released by malarial parasite in blood. HRP 2 is specific to *Plasmodium falciparum* and pLDH is specific to *P. vivax*. Parameters studied in this study are Hemoglobin (Hb), Total leukocyte count (TLC), Platelet count, Serum protein, Serum Bilirubin, Liver enzymes (SGPT, SGOT), Alkaline Phosphatase, Serum creatinine, Blood urea. Data were analyzed using SPSS software. The Erma PCE-525 five-part hematology analyzer and Erba XL-640 automated biochemistry analyzers provide rapid, standardized, and reproducible results with minimal operator-dependent variation, thereby

improving the reliability and accuracy of laboratory investigations. Automated internal quality control, high analytical precision, and reduced turnaround time facilitate early diagnosis and timely clinical decision-making in patients with malaria. However, automated analyzers may occasionally produce inaccurate results in samples with severe hemolysis, platelet clumping, lipemia, or instrument-related technical errors, necessitating correlation with peripheral blood smear examination and routine quality control procedures. Therefore, the combined use of automated analyzers with confirmatory microscopy and rapid malaria antigen testing has enhanced the overall diagnostic accuracy of our study.

Results

This study was undertaken to study effect of malaria on hematological and biochemical parameters. Total number of patients included in this study were 591 of which 475 patients were *P. Vivax* positive, 101 patients were *P. falciparum* positive and 15 patients showed mixed infection of both *P. Vivax* and *P. falciparum*. Mean age of the patient was 36 years. Youngest patient was 15 months old male child with *P. vivax* infection and eldest was 85 year old female with *P. falciparum* infection. Male to female ratio 7:1. Among the positive malaria patients, 520 were males and 71 were females. (Figure 1: Age distribution of the patients) (Figure 2: Sex Distribution of the patients) (Figure 3: Species Distribution of the patients)

Figure 1: Age Distribution of patients

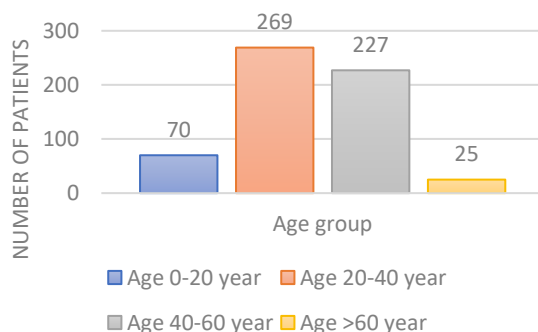


Figure 3 : Species distribution of patients

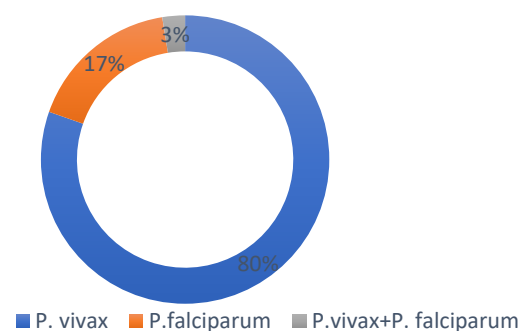
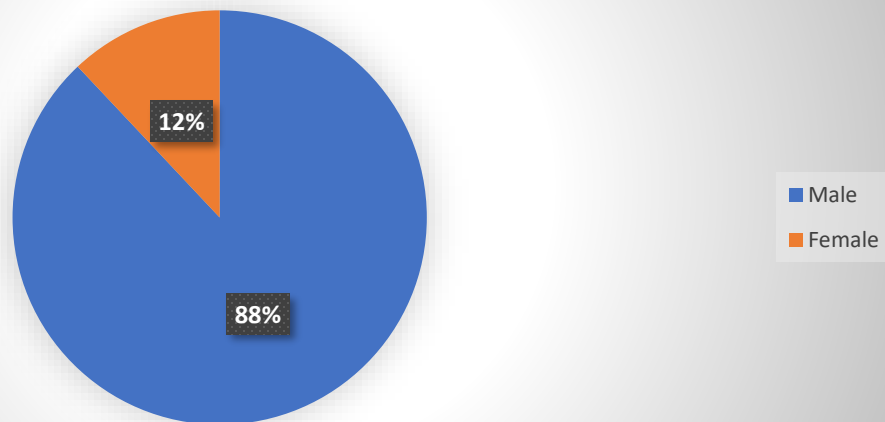


Figure 2: Sex distribution of patients



Thrombocytopenia was the most common hematological abnormality finding observed in malaria infection in the study. Thrombocytopenia defined as platelet count less than ($<$) $150,000/\mu\text{L}$ [5] was seen in 52 % of patients (309 patients) of which 259 patients were male and 50 were female. Out of 309 patients 255 patients were *P. vivax* positive and 44 patients were *P. falciparum* positive and 10 patients showed mixed infection of *P. vivax* and *P. falciparum*. Severe thrombocytopenia (platelet count $<50,000/\mu\text{L}$) was seen in 45% (263 patients) of the patients of which 225 patients were male and 38 were female. Out of 263 patients 206 patients were *P. vivax* positive and 54 patients were *P. falciparum* positive and 3 patients showed mixed infection of *P. vivax* and *P. falciparum*.

Anemia defined as hemoglobin (Hb) concentration <12 g/ dL for females and Hb <13

g/dL for males [5] was the second most frequently encountered hematological abnormality observed in malaria infection. Anemia was seen in 62%, (371 patients) of which 312 patients were male and 59 were female. Out of 371 patients 302 patients were *P. vivax* positive and 68 patients were *P. falciparum* positive and 1 patient showed mixed infection of *P. vivax* and *P. falciparum*. Leucopenia defined as total leukocyte count (TLC) $<4000/\mu\text{L}$ [5] was seen in 29 %, (174 patients) of which 155 patients were male and 19 were female, Out of 174 patients 95 patients were *P. vivax* positive and 76 patients were *P. falciparum* positive and 3 patients showed mixed infection of *P. vivax* and *P. falciparum*. Whereas leucocytosis defined as total leukocyte count (TLC) $>11000/\mu\text{L}$ [5] was present in minority of patients 2.5 %, (15 patients). (Table 1: Analysis of Hematological Parameters studied in the study patients)

Table 1: Analysis of Hematological Parameters in the study patients

Hematological alterations	P. vivax	P.falciparum	P. vivax + P.falciparum	Total
Number of patients	475	101	15	591
Normal platelet count ($>1,50,000$)	14	3	2	19 (3%)

Mild to moderate Thrombocytopenia (50,000 to 1,50000)	255	44	10	309 (52%)
Severe Thrombocytopenia (<50000)	206	54	03	263 (45%)
Anemia	302	68	01	371 (62%)
Leucopenia	95	76	03	174(29%)

Biochemical parameters

Biochemical parameters are valuable indicators of hepatic and renal involvement in malaria and aid in assessing disease severity. In healthy adults, the normal reference ranges are as follows: total bilirubin 0.3–1.2 mg/dL, direct (conjugated) bilirubin 0–0.3 mg/dL, blood urea 15–40 mg/dL, serum creatinine 0.6–1.3 mg/dL, serum glutamic oxaloacetic transaminase (SGOT/AST) 10–40 U/L, serum glutamic pyruvic transaminase (SGPT/ALT) 7–56 U/L, alkaline phosphatase (ALP) 44–147 U/L, and total serum protein 6.0–8.3 g/dL. Values above the upper reference limit for bilirubin, urea, creatinine, SGOT, SGPT, and alkaline phosphatase, and values below the lower reference limit for total serum protein, were considered abnormal in the present study. These biochemical alterations were evaluated to determine the extent of hepatic and renal dysfunction associated with malaria. [6,7] In terms of biochemical parameters, most common finding is elevated total bilirubin with majority patients showing raised direct bilirubin. Direct bilirubin (conjugated bilirubin) is the water-soluble form of bilirubin produced after unconjugated bilirubin is conjugated with glucuronic acid in hepatocytes by the enzyme uridine diphosphate-glucuronosyltransferase (UGT1A1). It is normally excreted into the bile and eliminated through the intestine. An increase in direct bilirubin indicates impaired hepatic excretion or cholestasis due to hepatocellular injury or biliary obstruction. In malaria, elevated direct bilirubin reflects hepatic dysfunction caused by parasitic infection, inflammatory cytokine-mediated

hepatocellular damage, intrahepatic cholestasis, and, to a lesser extent, hemolysis. Therefore, raised direct bilirubin serves as an important biochemical marker of liver involvement and disease severity, particularly in severe *Plasmodium falciparum* infection, although it may also be observed in *Plasmodium vivax* malaria [7]. Other biochemical parameters showing elevated values are urea, serum creatinine, SGOT, SGPT and alkaline phosphatase. Total serum protein level was found to be significantly decreased.

In our study total Bilirubin was raised in 505 patients (85%) of which 459 were male and 46 were female. Out of 505 patients 368 patients were *P. vivax* positive and 129 patients were *P. falciparum* positive and 8 patients showed mixed infection of *P. vivax* and *P. falciparum*. Direct bilirubin was raised in 518 patients (88%) of which 461 were male and 57 were female. Out of 518 patients 315 patients were *P. vivax* positive and 196 patients were *P. falciparum* positive and 7 patients showed mixed infection of *P. vivax* and *P. falciparum*. Blood urea level was raised in 213 patients (36%) of which 207 were male and 6 were female. Out of 213 patients 176 patients were *P. vivax* positive and 35 patients were *P. falciparum* positive and 2 patients showed mixed infection of *P. vivax* and *P. falciparum*. Serum creatinine was raised in 154 patients (26%) of which 131 were male and 33 were female. Out of 154 patients 99 patients were *P. vivax* positive and 50 patients were *P. falciparum* positive and 5 patients showed mixed infection of *P. vivax* and *P. falciparum*. Raised SGOT was seen in 136 patients (23%) of

which 133 were male and 03 were female. Out of 136 patients 98 patients were P. vivax positive and 24 patients were P. falciparum positive and 1 patient showed mixed infection of P. vivax and P. falciparum. Raised SGPT was seen in 98 patients (16%) of which 94 were male and 04 were female. Out of 98 patients 75 patients were P. vivax positive and 21 patients were P. falciparum positive and 2 patients showed mixed infection of P. vivax and P. falciparum. Raised Alkaline Phosphatase level was seen in 86 patients (14%) of which 85 were

male and 01 was female. Out of 86 patients 67 of them were P. vivax positive and 15 patients were P. falciparum positive and 3 patients showed mixed infection of P. vivax and P. falciparum. Serum protein was reduced in 266 patients (45%) of which 250 were male and 16 were female. Out of 266 patients 128 of them were P. vivax positive and 77 patients were P. falciparum positive and 1 patient showed mixed infection of P. vivax and P. falciparum. (Table 2: Analysis of Biochemical Parameters studied in the study patients)

Table 2: Analysis of Biochemical Parameters studied in the study patients

Abnormal Biochemical Parameters	P. vivax	P. falciparum	P. vivax + P. falciparum	Total
Number of patients	475	101	15	591
Direct Bilirubin (Raised)	315	196	7	518 (88%)
Serum Total Bilirubin (Raised)	368	129	8	505(85%)
Serum protein level (Decreased)	128	77	1	266 (45%)
Blood Urea (Raised)	176	35	2	213 (36%)
Serum creatinine (Raised)	99	50	5	154 (26%)
SGOT (Raised)	98	24	1	136 (23%)
SGPT (Raised)	75	21	2	98 (16%)
Alkaline phosphatase (Raised)	67	15	3	86 (14%)

DISCUSSION

The latest data from the World Health Organization (WHO) in 2023 indicate that there were approximately 249 million instances of malaria across 85 countries where the disease was prevalent. Notably, India and Indonesia were responsible for 94% of malaria related fatalities within the South-East Asia Region. Microscopy by peripheral smear examination has a sensitivity of approximately 90–95% and specificity of 95–100% when performed by experienced personnel, whereas RDTs demonstrate a sensitivity of 85–95% and specificity of 90–98%, with generally high

positive and negative predictive values in endemic settings. Molecular methods such as polymerase chain reaction (PCR) offer the highest sensitivity and specificity (>95%) and are useful for detecting mixed infections and low-level parasitemia, although their routine use is limited by cost and infrastructure requirements. ^[8]In India, urban cities such as Mumbai experience mostly seasonal outbreaks during and after monsoon rains, predominantly by P.Vivax. Dhawan et al., in a Mumbai-based community study, observed that mosquito transmission was high, highlighting the need

for stronger preventive education in urban areas^[9] Malaria in India is mainly caused by *Plasmodium vivax* and *Plasmodium falciparum*, the two most common species. Transmission occurs through the bite of infected female *Anopheles* mosquitoes. After inoculation, parasites invade hepatocytes and later erythrocytes, causing cyclical hemolysis, fever with chills, anemia, splenomegaly, thrombocytopenia, and inflammatory cytokine release. Anemia in malaria is multifactorial in origin. These factors include hemolysis of parasitized red blood cells, splenic and reticuloendothelial hyperactivity with a recent study revealing a role of interleukins (IL-4) and interferon's (IFN- γ) in erythropoietin suppression.^[9,10,11,12] In *falciparum* malaria, sequestration of parasitized red cells in microvasculature can lead to organ dysfunction and severe complications. According to Anvikar et al., *P. vivax* remains an important cause of recurrent malaria in India because dormant liver hypnozoites can reactivate and cause relapse.^[13] The disease affects all age groups, but children under five and pregnant women are at higher risk of developing complications from malaria.

The evaluation of the hematological and biochemical parameters plays an important role in understanding the pathophysiology of malaria and its systemic effects. Clinical complications include severe anemia, jaundice, acute kidney injury, cerebral malaria, seizures, pulmonary edema, metabolic acidosis, shock, disseminated intravascular coagulation, and multiorgan failure. Pregnant women may develop maternal anemia, fetal growth restriction, abortion, or low-birth-weight neonates. Diagnosis is based on peripheral smear microscopy (thick and thin smear), rapid diagnostic tests (RDTs) detecting parasite antigens, complete blood count, liver function tests, renal function tests, serum electrolytes, blood glucose, coagulation profile, and urine analysis in severe disease.

Anemia was observed in the majority (62%) of patients, attributed to hemolysis, bone marrow suppression and sequestration of infected

erythrocytes. Similar findings were reported in a study by Sinha et al in which anemia was reported in 78 % of malaria patients, while Shah et al study reported anemia in 53 % of patients.^[14,15]

Thrombocytopenia was the most consistent hematological abnormality and is considered as a hallmark of malaria. The mechanism includes immune-mediated destruction and splenic sequestration. A study from Shah et al reported thrombocytopenia in 84 % of patients, while another study by Surve et al reported a prevalence of approximately 71%.^[15,16]

Leucopenia was observed in 29% of patients in this study, which is consistent with study done by Shah et al reporting leucopenia in approximately 20–30% of patients. Most Indian studies have shown that total leukocyte count remains normal or low in malaria, while leukocytosis is uncommon and usually associated with severe disease or secondary infections.^[15]

Among biochemical parameters hyperbilirubinemia was the most prominent biochemical abnormality in this study, with elevated total bilirubin in 78% and direct bilirubin in 88% of patients, a study by Sinha et al. reported unconjugated hyperbilirubinemia in 33.8% of patients. The higher prevalence in this study suggests more pronounced hepatic involvement, possibly due to higher parasite burden or mixed infections.^[14]

Elevated SGOT, SGPT liver enzymes indicate hepatic involvement. A study done by Khuriya et al similarly reported mild to moderate elevation of transaminases, reflecting hepatocellular injury due to parasite invasion and systemic inflammation.^[17]

Renal dysfunction, indicated by elevated blood urea (36%) and serum creatinine (26%), was observed in this study. Comparable findings have been reported in Khuriya et al study, where renal involvement was noted in approximately 29% of patients. Acute kidney injury in malaria is primarily associated with *falciparum* infection and results from hemoglobinuria, microvascular obstruction, and hypovolemia.^[17]

Hypoproteinemia was observed in 45% of patients in this study. Although not extensively studied in Indian literature, reduced serum protein levels have been attributed to hepatic dysfunction, increased catabolism, and vascular leakage during systemic infection.

Prediction of the hematological changes in malaria enables the clinician for early therapeutic intervention to prevent the major complications. Haematology parameters can help to provide a presumptive treatment, especially when the results of the parasitological examination are not immediately available or are uncertain to decide treatment for malaria and help to intensively care for the patient and prevent complications. [17,18,19]

The hematological and biochemical abnormalities observed in malaria reflect the underlying pathophysiological changes caused by parasitic infection. Decreased hemoglobin indicates hemolysis of parasitized and non-parasitized erythrocytes, splenic sequestration, and suppressed erythropoiesis. Thrombocytopenia results from immune-mediated platelet destruction, splenic pooling, and increased peripheral consumption, while leukopenia is associated with splenic sequestration and redistribution of leukocytes. Conversely, leukocytosis may indicate severe infection, secondary bacterial infection, or an exaggerated inflammatory response. Elevated

serum bilirubin, particularly direct bilirubin, along with increased SGOT, SGPT, and alkaline phosphatase, reflects hepatocellular injury and intrahepatic cholestasis. Increased blood urea and serum creatinine indicate renal impairment due to dehydration, hemoglobinuria, acute tubular injury or severe malaria. Decreased total serum protein suggests impaired hepatic synthetic function, increased catabolism, malnutrition, or capillary leakage during systemic inflammation. Collectively, these laboratory abnormalities provide important diagnostic and prognostic information regarding disease severity and organ involvement in malaria.

Conclusion

Malaria is associated with significant hematological and biochemical changes. Anemia and thrombocytopenia are key hematological indicators. Hyperbilirubinemia and abnormal liver and renal function tests are common biochemical findings. Combined evaluation aids in early diagnosis and prognosis. The analysis of these hematological and biochemical parameters helps clinicians to differentiate malaria from other febrile illnesses, especially in endemic regions where overlapping clinical features are common. This helps in improving the diagnostic accuracy, patient management and prevent complications related to organ dysfunction.

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