

A Retrospective Cross Sectional Study on the Hematological impact of Malaria and the significance of various blood parameters in the detection of Malaria

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ABSTRACT

Objective: To evaluate the significance of hematological parameters in the diagnosis of malaria and estimate the frequency of malaria at a tertiary care center in Karachi, Pakistan.

Study Design: Cross-Sectional study.

Place and Duration: This Study was conducted at the Pathology Laboratory of Creek General Hospital, Karachi from 15th February, 2024 to 31st May, 2024.

Methodology: Data from 293 malaria-positive blood samples, confirmed via rapid diagnostic test, was analyzed. Simple random sampling was used to retrieve data from Laboratory Information Management System (LIMS) after approval of ethical review board, from 1st December 2023 to 15th May 2024. Males and females of all age groups were included, excluding co-infection with dengue and other haemorrhagic fevers. Haematological parameters were obtained from complete blood count reports and Plasmodium was detected on peripheral smears. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 23, with a p-Value <0.05.

Results: Plasmodium was confirmed microscopically in 69.3% cases, with 14.8% Plasmodium falciparum and 40.9% Plasmodium vivax. Plasmodium Falciparum patients had lower red blood cell, white blood cell and platelet counts but higher red cell parameters compared to Plasmodium vivax. Mean lymphocyte count was higher in Plasmodium falciparum. Thrombocytopenia was more frequent in Plasmodium vivax, with lower platelet counts in Plasmodium falciparum. Lymphocyte count and thrombocytopenia correlated with Plasmodium infection (p<0.05).

Conclusion: Plasmodium vivax is more common than Plasmodium falciparum infection. Variations in lymphocyte and platelet counts with different species of Plasmodium can assist in provisional diagnosis, in resource-limited settings.

Keywords: Haematological profile, Malaria, Plasmodium falciparum, Plasmodium vivax, Rapid diagnostic test, Thick and thin smear.

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INTRODUCTION

Malaria is a major global health concern due to its high mortality and morbidity rates.¹ It is a life-threatening disease caused by parasites of the Plasmodium species, which are transmitted to humans through the bites of infected Anopheles mosquitoes.² Plasmodium falciparum (P. falciparum) and Plasmodium vivax (P. vivax) are the most dangerous species.³ Malaria is responsible for 249 million cases and 608,000 deaths across the world.^{4,5} Pakistan ranks seventh among Eastern Mediterranean countries that collectively bear 98% of the malaria burden.⁴⁻⁷

Diagnosing malaria presents a multifaceted challenge, especially in areas where the disease is endemic. The acute febrile illness resulting from Plasmodium infection is difficult to differentiate from other febrile illnesses resulting in late detection and multiple complications.⁵ The gold standard for diagnosis of this disease has traditionally been microscopic examination of peripheral blood smears, requiring skilled personnel to accurately identify the parasite species.⁸ However, this method has limitations, particularly in cases of low parasitemia or when expertise is scarce. In recent years, there has been growing interest in utilizing blood parameters obtained from complete blood count (CBC) reports as adjunctive tools for the detection

of malaria as this infection causes abnormalities in hematological indices giving potential clues for diagnosis. CBC is a widely available, cost-effective, and rapid test that provides valuable information about various blood components, including red blood cells, white blood cells, and platelets.⁹ Previous studies have highlighted the association between malaria and alterations in blood parameters.^{9,10} Hematological abnormalities such as anemia, thrombocytopenia, and lymphocytosis assist in clinical suspicion of malaria even in the absence of positive smears resulting in early detection of the disease.^{11,12} In this study, we aim to assess the frequency of malarial infection and the importance of blood parameters in the diagnosis of malaria with the rationale that the hematological abnormalities in different malaria-endemic settings show specific patterns, reflecting variations in the parasite species, host factors, and disease severity.^{8,12} Identifying the burden of disease and recognizing the changes in blood parameters will help in early detection of the illness and complications such as severe anemia, bleeding, dehydration etc. By evaluating the relationship between malarial infection and blood parameters, our results may contribute to the development of more accessible and reliable diagnostic strategies for malaria, thereby promoting timely and effective management of the disease.

METHODOLOGY

This retrospective cross sectional study was conducted at the Pathology Laboratory of Creek General Hospital (CGH), Karachi from 15th February, 2024 to 31st May, 2024 after taking permission from the Institutional review board Reference no: CGH/Ethics/2024/14/02/345, dated 14th February 2024.

This study analyzed the data of 293 blood samples, positive for malaria on rapid diagnostic test. Data was retrieved by simple random sampling technique from laboratory information management system (LIMS) from 1st December 2023 to 15th May 2024. Cases included both males and females of all age groups. Cases of co-infection with dengue and other haemorrhagic fever were excluded. Haematological parameters including white blood cells (WBC), red blood cells (RBC), platelets, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and hemoglobin levels (Hb) of malaria positive cases were collected from complete blood count (CBC) reports. CBC of these cases had been performed previously by a trained laboratory technician on haematology analyzer Sysmex XNL- 350 after blinding and checking the controls.² The data was meticulously recorded by a pathologist on a provided proforma. Rapid diagnostic test (RDT) for malaria was performed earlier using the malaria *Plasmodium falciparum/Plasmodium vivax*. The antigen (Ag) Rapid Test Cassette (Abbott) with a relative sensitivity of 100% and specificity of 98.7%. Thick and thin blood smears of these samples, stained with Leishman stain as per protocol of Diagnostic Chemicals (Dia Chem) were reviewed under a microscope at 40X and 100X by two pathologists and malarial parasite was identified. The presence of malarial parasites was confirmed on thick smear and a thin blood smear

was prepared for the identification of *Plasmodium* species.

Data Analysis: Statistical analysis was performed using SPSS version 23, with a confidence limit/p-Value set at ≤ 0.05 . The frequency of *Plasmodium* was calculated in various age groups and in both males and females. Mean and standard deviation (SD) were calculated for numerical variables such as age, Hemoglobin (Hb), blood cell counts including red blood cells (RBC), platelets, monocytes, lymphocytes, eosinophils and neutrophils, MCH, MCV and MCHC. Percentage and frequency were calculated for gender and species of malarial parasite. To evaluate the significance of blood parameters in the diagnosis of malaria, changes in white blood cells (WBC), and platelets, RBC, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and Hb level among different *Plasmodium* species were assessed using the two sample t- test.

RESULTS

Malarial parasite was positive in 12.74% (n=293) cases on rapid diagnostic test (RDT). These included 67.4% male patients and 32.6% females. The mean age value was 30.6 ± 17.2 years. The maximum number of malaria positive cases, 36% (n=105) was seen in the 11-25 years age group. On RDT, *P. vivax* was detected in 82.6% cases (n=242), whereas *P. falciparum* was detected in 17.1% samples (n=50) and co-infection of *P. falciparum* and *P. vivax* was observed in 0.3% (n=1) (Figure 1).

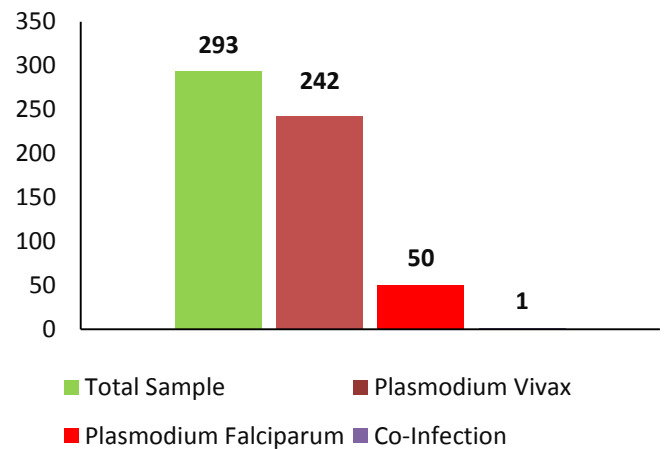


Figure 1: Frequency of Plasmodium on rapid diagnostic test (RDT) (N=293)

On microscopic examination, malarial parasite was identified in 69.3% (n=203) cases. *P. vivax* infection was common in all age groups. *P. falciparum* was detected in 14.8% (n=30) smears in mainly two stages of development including ring form and gametocyte stage (Figure 2). *P. vivax* was seen in 40.9% (n=83) smears. Rest of the smears, 44.3% (n=90) did not show the parasite. Majority 34.3% (n=83) cases of *P. vivax* infection were seen in the month of August while most 40% (n=20) cases of *P. falciparum* infection were observed in September during the monsoon season (Figure 3).



Figure 2: Gametocyte of *Plasmodium falciparum* at 40X on peripheral smear. (N=293)

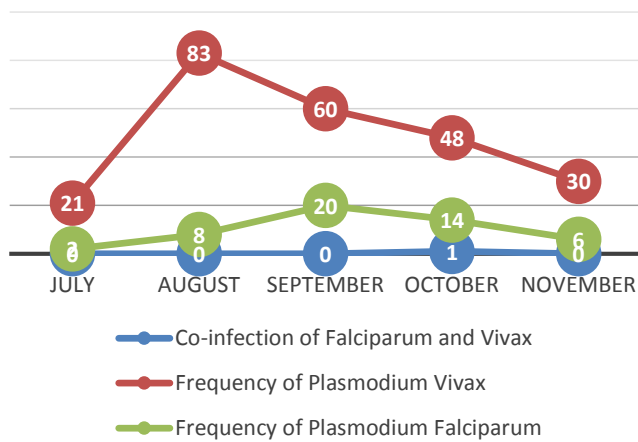


Figure 3: Trend analysis of malarial parasite on rapid diagnostic test. (N=293)

Table I: Mean values, ranges and P values of blood parameters in *Plasmodium vivax* and *Plasmodium falciparum* infection. (N=293)

Parameters	Expected Range	Plasmodium Vivax (n=242)	Plasmodium Falciparum (n=50)	Value of t-test	p-Value
		Mean (SD)	Mean (SD)		
RBC	3.00-6.00	4.30 (0.74)	4.25 (0.89)	0.27	0.789 (p > 0.05)
Hb	12.0-16.0	11.90 (2.18)	11.83 (2.52)	0.41	0.683 (p > 0.05)
PLT	100-300	83.34 (48.76)	76.18 (81.33)	2.06	0.041 (p < 0.05)
TLC	4.0–10.0	5.80 (6.83)	3.87 (6.56)	0.95	0.345 (p > 0.05)
Neutrophils	50.0-70.0	72.46 (13.76)	68.53 (17.63)	1.92	0.058 (p > 0.05)
Lymphocytes	20.0–40.0	44.46 (12.49)	56.11 (16.27)	2.03	0.047 (p < 0.05)
Eosinophils	1.00-4.00	2.40 (1.22)	2.55 (1.00)	0.51	0.607 (p > 0.05)
Monocytes	1.0–15.0	3.63 (1.68)	3.76 (1.54)	0.74	0.458 (p > 0.05)
PCV	11.5-14.5	35.81 (6.39)	34.90 (7.50)	0.76	0.447 (p > 0.05)
MCH	20.0-40.0	27.46 (3.4)	27.93 (4.77)	0.41	0.686 (p > 0.05)
MCHC	30-40	32.78 (1.44)	33.37 (1.44)	2.01	0.046 (p < 0.05)
MCV	80-100	82.37 (9.6)	82.97 (12.98)	0.05	0.96 (p > 0.05)

The mean hematological values and p-Values of the blood parameters are shown in Table-I. The p-values for each parameter were computed to assess the differences between the *Plasmodium vivax* and *Plasmodium falciparum* groups. For each parameter, group means and standard deviations (SDs) were calculated to summarize the central tendency and variability, respectively. Group variances were estimated based on the SDs of each group. Mean values of RBC, WBC and platelets were relatively lower in patients with *P.falciparum* malaria compared to those with *P.vivax* malaria. The p-Value of platelet count was 0.041 (which is <0.05 and statistically significant). The mean values of MCV, MCH and PCV showed no significant changes in malarial infection. Only MCHC was significant with a p-value of 0.046 (which is <0.05 and statistically significant) as shown in Table-I. There was no significant change in the mean value of hemoglobin in patients with *P.falciparum* malaria compared to *P.vivax* as shown in Table-I. The p-value was 0.683 (which is >0.05 and not statistically significant).

Leukocytosis was seen in 1.4% (n=4) cases and leucopenia was seen in 1.02% (n=3) cases. 6.14% (n=18) cases of *P.vivax* infection showed neutrophilia. 17.7% (n=52) cases showed lymphocytosis. The mean lymphocyte count was higher in patients with *P.falciparum* compared to those with *P. vivax* infection and p-value was 0.047 (which is <0.05 and significant). Thrombocytopenia was observed in 71% (n=208) cases out of which 80.8% (n=168) were in *P.vivax* infection and 19.2% (n=40) in *P.falciparum* infection. No case of thrombocytosis was observed. Two sample t-test revealed a significant relationship between MCHC and *Plasmodium* infection. Variation in lymphocyte count and thrombocytes was also significant.

DISCUSSION

According to the World Health Organization (WHO), severe cases of malaria can be identified through changes in blood chemistry and hematological parameters.¹³

In this study, the frequency of malarial infection on rapid diagnostic test (RDT) was 12.74% and on microscopic examination, it was 69.3% including 14.8% *P. falciparum* and 40.9% *P. vivax* infection. However, another study conducted in Pakistan had an overall malaria prevalence of 23.3%, including 16.29% *P. falciparum* and 79.13% *P. vivax*.⁷ RDT sensitivity varies depending on the malaria species and levels of parasitemia. These tests are designed to detect specific antigens produced by malaria parasites and they might be effective at detecting one species more than the other. Genetic variations in the *Plasmodium* species may affect the antigens that RDTs are designed to detect.¹⁴ Factors such as storage conditions, expiration date, and handling of the kits can affect their performance. RDTs might also be less sensitive in detecting low levels of parasites compared to microscopy as their performance can be affected by the density of parasites in the blood. Peripheral smears, especially when performed by skilled technicians, can detect lower levels of parasites and are necessary for the confirmatory diagnosis and species identification as RDTs can give false positive results due to cross reactivity.¹⁴

Anemia is a frequent complication of malaria.¹⁵ However, there was no significant change in the mean of hemoglobin concentration between *P. falciparum* (11.83 ±2.52) and *P. vivax* (11.90 ±2.18) infection and anemia was not associated with *Plasmodium* infection in our study. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and packed cell volume (PCV) showed no significant change between *P. falciparum* and *P. vivax* infected patients in this study. Only mean corpuscular hemoglobin concentration (MCHC) was significant. Variable rates of anemia have been reported in malarial infection in other studies.¹⁶⁻¹⁸ The prevalence and strain of malarial parasite and severity of the disease can influence the association of anemia with malarial parasite.^{17,18} In contrast to this study, anemia showed strong association with *P. falciparum* infection in other studies.^{15,19} Ahmed et al reported significantly lower values of Hb concentration, RBC count, MCV, MCH and MCHC in *P. falciparum*-infected patients.¹⁹ Differences in sample size, demographic characteristics, variations in age, gender and nutritional status of the study population might account for these discrepancies, upon comparison with current study settings.

In our study, 18 (6.14%) cases, all of *P. vivax* infection showed neutrophilia similar to another study in which neutrophilia was more frequent in *P. vivax* infection (37.7%) than *P. falciparum* (21%).²⁰ However, neutrophilia was not associated with *Plasmodium* infection in this study. The p-value was 0.058 which is >0.05 and not statistically significant (Table 1). In another study conducted in Lahore observed neutrophilia in only 58 cases of malaria.²¹ Additionally, present study did not observe leukocytosis or leucopenia. In contrast, leucopenia was observed in 52.4% of *P. falciparum* and 44% of *P. vivax* cases in another study.²⁰ One study also reported leukocytosis in patients infected with malaria.¹⁷ The variability in the observed

hematological responses in malaria studies may be attributed to small sample size, differences in study populations, timing of blood collection relative to stage of infection, multifaceted nature of the immune response to malaria influenced by genetic factors and the severity of infection.^{14,16,18}

Lymphocytosis was significant in *Plasmodium* infection, with higher mean lymphocyte counts in *P. falciparum* compared to *P. vivax* infections. Our finding of lymphocytosis is similar to a study by Abdelnasser et al but in contrast to other studies that reported lymphopenia in both *P. falciparum* and *P. vivax* infections.^{11,20,22} The differences can be the result of demographic and geographic variations and the variable immune response depending on the severity of infection.¹⁴ The host immune response is more intense in *P. falciparum* infection as compared to *P. vivax* infection.^{15,16} No significant changes were found in the mean values of monocytes and eosinophils among malaria-infected patients.

Decrease in platelet count is a common hematological change observed in malaria patients.²² The mean platelet count was lower in *P. falciparum*-infected patients as compared to *P. vivax* infected patients. There was a statistically significant reduction in platelet count and p-value was 0.041 (which is <0.05) among malaria-infected patients, with thrombocytopenia occurring in 71% cases, more in *P. falciparum* infection as compared to *P. vivax* infection. Ahmed et al reported that thrombocytopenia was significantly associated with malaria.¹⁹ Another study by Awoke et al observed thrombocytopenia in 85% of *P. vivax* and 83% of *P. falciparum* malaria cases.²⁰ *P. falciparum* is generally more aggressive and has more severe pathogenic mechanisms compared to *P. vivax*.^{16,22} It can cause more extensive damage to the vascular endothelium leading to greater platelet activation and destruction. It triggers a stronger inflammatory response and also induces the production of platelet bound antibodies that target the platelets for destruction by the immune system. Bone marrow suppression is more severe with *P. falciparum* compared to *P. vivax* infection.^{21, 23, 24} Platelet clumps may also be miscounted as single platelets by the automated analyzer leading to pseudo-thrombocytopenia.^{22, 25}

Overall, our study highlights the significant variations in blood parameters in malaria infections, with thrombocytopenia and lymphocytosis being more prominent in *P. falciparum* infection.¹³ Thrombocytopenia could be due to high parasite burden or cytokine mediated destruction. Lymphocytosis in *P. falciparum* malaria may suggest a mild or resolving infection or an immune response in already exposed individuals. This study lacks clinical data due to which the association between hematological changes and clinical features, disease severity, complications, or patient recovery cannot be observed.

CONCLUSION

Plasmodium vivax is the more common species in this area. Blood parameters including lymphocyte count and platelet count show variable significant changes in malarial infection with different species of *Plasmodium*. Therefore, they can aid in provisional diagnosis of malaria in settings lacking conventional diagnostic tools.

RECOMMENDATIONS

- Early antimalarial therapy is recommended in endemic areas based on clinical suspicion. Severe changes indicate *P.falciparum* infection whereas, lymphocytosis may suggest mild infection, recovery or prior immunity.
- Blood parameters including lymphocyte and platelet count abnormalities can be used to screen for malaria in resource-limited settings

AUTHOR'S CONTRIBUTION

Shams M: Conceived the idea, data collection, manuscript writing, literature search and review

Iqbal M: Conceived the idea, data collection, designed the research methodology, manuscript writing

Hussain NH: Data analysis and data interpretation

Zaheer H: Literature review, data analysis, manuscript final reading

Pario S: Data analysis, manuscript final reading

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Conflict of Interest: None.

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