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Original Article

Hematological Changes in Malaria: A Comparative Study

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ABSTRACT

Background: Malaria remains an important cause of hematological morbidity in Pakistan, where species distribution and clinical presentation vary across regions and healthcare settings. Routine complete blood count and peripheral smear findings may provide useful supportive information in malaria evaluation, but local species-wise hematological data remain limited. **Objective:** To describe the spectrum of hematological abnormalities among confirmed malaria cases and compare selected parameters between *Plasmodium falciparum* and *Plasmodium vivax* infections. **Methods:** This comparative cross-sectional study was conducted at Pak-Emirates Military Hospital, Rawalpindi, from April 2022 to March 2024. Clinically suspected malaria patients were screened using peripheral blood smear microscopy and rapid diagnostic testing. Confirmed malaria-positive patients underwent complete blood count assessment, peripheral smear review, and reticulocyte count estimation. Species-wise comparisons were performed between *P. falciparum* and *P. vivax* mono-infections using appropriate statistical tests. **Results:** Among 400 clinically suspected patients, 74 (18.5%) were malaria-positive. *P. falciparum* was identified in 39 cases (52.7%), *P. vivax* in 27 (36.5%), and mixed infection in 8 (10.8%). Anemia was present in 64 patients (86.5%), while thrombocytopenia occurred in 53 (71.6%). Relative lymphopenia was more frequent in *P. falciparum* than *P. vivax* infection (33.3% vs. 11.1%; p<0.04). Clinically significant bleeding occurred in 3 of 53 thrombocytopenic patients (5.7%). **Conclusion:** Malaria was associated with frequent anemia, thrombocytopenia, and smear evidence of erythropoietic stress. Relative lymphopenia showed a species-wise association with *P. falciparum*, but prospective multicenter validation is needed before prognostic interpretation. **Keywords:** Malaria; *Plasmodium falciparum*; *Plasmodium vivax*; anemia; thrombocytopenia; lymphopenia; hematological changes; Pakistan.

INTRODCUTION

Malaria remains a major infectious disease burden in tropical and subtropical regions and continues to cause substantial morbidity in Pakistan, where climate variability, population movement, vector ecology, and uneven access to timely diagnosis contribute to persistent transmission. The Eastern Mediterranean Region continues to report a considerable malaria burden, with Pakistan contributing substantially to regional cases, particularly after the post-flood increase in transmission observed during 2022–2024 (1,2). Although national and regional surveillance studies frequently report *Plasmodium vivax* as the predominant species in many Pakistani settings, the relative distribution of *P. vivax*, *P. falciparum*, and mixed infections may vary by geography, referral pattern, season, population mobility, and hospital catchment characteristics (3). This variability is clinically important because malaria species may differ not only in epidemiological frequency but also in their hematological profile and potential clinical course.

Hematological abnormalities are among the most frequent laboratory manifestations of malaria and may assist clinicians in recognizing disease patterns, assessing clinical risk, and prioritizing monitoring in resource-limited settings. Malaria-associated anemia results from a combination of parasitized and non-parasitized erythrocyte destruction, splenic clearance, immune-mediated hemolysis, inflammatory suppression of erythropoiesis, and altered iron regulation. Thrombocytopenia is also common and may occur through immune-mediated platelet destruction, splenic sequestration, platelet activation, and marrow suppression. Leukocyte abnormalities, including leukopenia, neutrophil changes, monocytosis, atypical lymphocytes, and lymphopenia, may reflect host inflammatory response and immune-cell redistribution during acute infection. In pediatric and severe malaria cohorts from Pakistan, anemia and thrombocytopenia have been repeatedly identified as frequent complications, but species-specific hematological patterns remain incompletely characterized across different local clinical settings (4,5).

Although *P. falciparum* has traditionally been associated with more severe systemic disease, increasing evidence indicates that *P. vivax* may also produce clinically significant hematological morbidity. Therefore, assumptions that *P. vivax* infection is uniformly mild may underestimate its contribution to anemia, thrombocytopenia, and other laboratory abnormalities. At the same time, routine complete blood count parameters are widely available in Pakistani hospitals and may provide practical supportive information before or alongside species confirmation. However, the interpretation of these parameters requires locally generated evidence, because hematological findings can be influenced by patient demographics, nutritional status, background anemia prevalence, comorbid infections, referral bias, and parasite species distribution.

Existing literature from Pakistan provides valuable information on malaria epidemiology and selected hematological complications, but there remains a need for hospital-based comparative data describing the spectrum of complete blood count and peripheral smear abnormalities among confirmed malaria patients, particularly with direct comparison between *P. falciparum* and *P. vivax* infections. Such evidence may help clarify whether specific hematological patterns are more frequently associated with one species than another and may support more careful clinical interpretation of routine laboratory findings. The present study was therefore conducted to describe hematological abnormalities among confirmed malaria cases presenting to a tertiary-care hospital in Rawalpindi and to compare selected hematological parameters between *P. falciparum* and *P. vivax* infections. The primary objective was to determine the frequency and pattern of anemia, thrombocytopenia, leukocyte abnormalities, peripheral smear morphology, and reticulocyte response among malaria-positive patients, with species-wise comparison where applicable.

MATERIAL AND METHODS

This comparative cross-sectional study was conducted in the Department of Medicine, Pak-Emirates Military Hospital, Rawalpindi, Pakistan, over a two-year period from April 2022 to March 2024. The study was designed to evaluate the hematological profile of laboratory-confirmed malaria cases and to compare selected hematological abnormalities between patients infected with *Plasmodium falciparum* and those infected with *Plasmodium vivax*. A consecutive sampling approach was used, and all clinically suspected malaria cases presenting during the study period were screened for eligibility. Clinically suspected malaria was defined as acute febrile illness with temperature ≥38°C accompanied by one or more compatible features, including chills, rigors, headache, myalgia, or splenomegaly, in the absence of another confirmed diagnosis at initial presentation.

Patients of either sex and all eligible age groups presenting with clinical suspicion of malaria were considered for enrollment. Patients were excluded if they had laboratory or clinical evidence suggestive of dengue fever, chronic liver disease, inherited or acquired bleeding disorders, pre-existing thrombocytopenia, chronic hematological malignancy, autoimmune disease, or any other condition likely to independently alter blood cell parameters. Patients with recent intake of drugs known to affect

hematological indices, including anticoagulants, antiplatelet agents, cytotoxic drugs, or other marrow-suppressive medications, were also excluded. Written informed consent was obtained from adult participants and from parents or legal guardians of pediatric participants before enrollment. The study was conducted after institutional ethical approval, and participant confidentiality was maintained throughout data collection and analysis.

All enrolled clinically suspected cases underwent malaria testing using peripheral blood smear microscopy and immunochromatographic rapid diagnostic testing. Thick and thin peripheral blood smears were prepared and stained with Leishman's stain. Thick smears were examined for parasite detection and density estimation, while thin smears were used for species identification and morphological assessment. Rapid diagnostic testing was performed using antigens targeting histidine-rich protein-2 for *P. falciparum* and pan-*Plasmodium* lactate dehydrogenase for non-falciparum infection. A case was classified as malaria-positive when microscopy and/or rapid diagnostic testing showed evidence of malaria infection. Species classification was based on smear morphology and rapid diagnostic test pattern, with independent review of malaria-positive smears by two trained microscopists. Mixed infection was recorded when evidence of both *P. falciparum* and *P. vivax* was identified. For the main species-wise comparison, *P. falciparum* and *P. vivax* mono-infections were compared separately, while mixed infections were described as a distinct group to avoid misclassification in direct interspecies analysis.

Venous blood samples were collected under aseptic precautions from confirmed malaria-positive patients for complete blood count, peripheral smear review, and reticulocyte count estimation. Complete blood count parameters included hemoglobin concentration, total leukocyte count, platelet count, red cell indices, and automated differential leukocyte count, measured using the Sysmex XN-1000 automated hematology analyzer. Peripheral smears were reviewed for red cell morphology, platelet adequacy, nucleated red blood cells, hypersegmented neutrophils, toxic granulation, atypical lymphocytes, and other morphological abnormalities. Reticulocyte count was assessed to evaluate erythropoietic response. Parasite density was calculated on thick smear per 200 white blood cells and expressed as parasites per microliter of blood.

The primary variables were malaria positivity, parasite species, anemia, thrombocytopenia, total leukocyte count abnormality, neutrophil abnormality, relative lymphopenia, monocytosis, eosinophil abnormality, basophil abnormality, peripheral smear morphology, nucleated red blood cells, atypical lymphocytes, toxic granulation, and elevated reticulocyte count. Anemia was defined as hemoglobin below the age- and sex-appropriate lower reference threshold used by the institutional laboratory. Thrombocytopenia was defined as platelet count <150,000/µL. Total leukocyte count was categorized as leukopenia when <4,000/µL, normal when 4,000–11,000/µL, and leukocytosis when >11,000/µL. Relative neutrophil percentage was categorized as neutropenia when <40%, normal when 40%–70%, and neutrophilia when >70%. Relative lymphopenia was defined as lymphocyte percentage <20%, normal lymphocyte proportion as 20%–40%, and lymphocytosis as >40%. Monocytosis was defined as monocyte percentage >8%, eosinophilia as eosinophil percentage >4%, and basophilia as basophil percentage >1%. Clinically significant bleeding was recorded when the patient had observable hemorrhagic manifestations temporally associated with thrombocytopenia.

To reduce measurement bias, malaria-positive peripheral smears were reviewed independently by two trained microscopists for species confirmation, parasite estimation, and smear morphology. Automated hematology results were interpreted alongside manual smear review where clinically relevant. Patients with major pre-existing hematological or systemic conditions likely to confound blood-cell parameters were excluded. Data were checked for completeness, consistency of denominators, and plausibility before analysis. Mixed infections were retained in descriptive analyses but not combined with either mono-infection group for primary *P. falciparum* versus *P. vivax* comparisons.

Data were entered into Epi Info version 7.2 and analyzed using IBM SPSS Statistics version 26.0. Categorical variables were summarized as frequencies and percentages. Continuous variables were assessed for distribution using the Shapiro–Wilk test and summarized as mean ± standard deviation for normally distributed data or median with interquartile range for non-normally distributed data. Comparisons between *P. falciparum* and *P. vivax* mono-infection groups were performed using the independent-samples t-test or Mann–Whitney U test for continuous variables, depending on distribution, and the Chi-square test or Fisher's exact test for categorical variables, depending on expected cell counts. A two-tailed p-value <0.05 was considered statistically significant. Exact p-values were planned for all inferential comparisons. Analyses were interpreted cautiously because of the cross-sectional design and limited number of malaria-positive cases, and findings were considered species-associated hematological differences rather than evidence of causality or validated prognostic performance.

RESULTS

A total of 400 clinically suspected malaria patients were screened during the study period. Of these, 74 patients were confirmed positive for malaria by peripheral blood smear microscopy and/or rapid diagnostic testing, yielding a malaria positivity rate of 18.5%. Among confirmed malaria-positive cases, *Plasmodium falciparum* was identified in 39 patients, *Plasmodium vivax* in 27 patients, and mixed *P. falciparum* plus *P. vivax* infection in 8 patients.

Table 1. Malaria Detection and Species Distribution Among Clinically Suspected Cases

Diagnostic Category	n	%
Total clinically suspected cases screened	400	100.0
Malaria-positive cases	74	18.5
Malaria-negative cases	326	81.5
***P. falciparum* among malaria-positive cases**	39	52.7
***P. vivax* among malaria-positive cases**	27	36.5
Mixed infection among malaria-positive cases	8	10.8

Among the 74 confirmed malaria-positive patients, *P. falciparum* represented the largest species group, accounting for 52.7% of infections, followed by *P. vivax* at 36.5%. Mixed infection was identified in 10.8% of malaria-positive cases. The confirmed malaria positivity rate among clinically suspected patients was 18.5%.

Table 2. Key Hematological Abnormalities Among Malaria-Positive Patients by Species

Hematological Parameter	Total n/N (%)	*P. falciparum* n/N (%)	*P. vivax* n/N (%)	Mixed Infection n/N (%)
Anemia	64/74 (86.5)	35/39 (89.7)	23/27 (85.2)	6/8 (75.0)
Normal total leukocyte count	60/74 (81.1)	31/39 (79.5)	23/27 (85.2)	6/8 (75.0)
Leukocytosis	8/74 (10.8)	5/39 (12.8)	2/27 (7.4)	1/8 (12.5)
Leukopenia	6/74 (8.1)	3/39 (7.7)	2/27 (7.4)	1/8 (12.5)
Normal neutrophil proportion	63/74 (85.1)	32/39 (82.1)	24/27 (88.9)	7/8 (87.5)
Neutrophilia	9/74 (12.2)	6/39 (15.4)	2/27 (7.4)	1/8 (12.5)
Neutropenia	2/74 (2.7)	1/39 (2.6)	1/27 (3.7)	0/8 (0.0)
Normal lymphocyte proportion	51/74 (68.9)	24/39 (61.5)	22/27 (81.5)	5/8 (62.5)
Relative lymphopenia	18/74 (24.3)	13/39 (33.3)	3/27 (11.1)	2/8 (25.0)
Relative lymphocytosis	5/74 (6.8)	2/39 (5.1)	2/27 (7.4)	1/8 (12.5)
Thrombocytopenia	53/74 (71.6)	31/39 (79.5)	16/27 (59.3)	6/8 (75.0)
Thrombocytopenia with clinically significant bleeding	3/53 (5.7)	2/31 (6.5)	1/16 (6.3)	0/6 (0.0)
Thrombocytopenia without clinically significant bleeding	50/53 (94.3)	29/31 (93.5)	15/16 (93.8)	6/6 (100.0)

Anemia was the most frequent hematological abnormality, affecting 86.5% of malaria-positive patients. The frequency of anemia was numerically higher in *P. falciparum* infection than in *P. vivax* infection, but the difference was small. Most patients had a normal total leukocyte count, while leukocytosis and leukopenia were observed in 10.8% and 8.1% of malaria-positive cases, respectively. Relative lymphopenia was more frequent in *P. falciparum* infection than in *P. vivax* infection. Thrombocytopenia

was common, affecting 71.6% of malaria-positive patients, and was numerically more frequent in *P. falciparum* infection than in *P. vivax* infection. Despite the high frequency of thrombocytopenia, clinically significant bleeding was uncommon and occurred in only 5.7% of thrombocytopenic patients.

Table 3. Peripheral Blood Smear and Reticulocyte Findings Among Malaria-Positive Patients

Finding	Total n/N (%)	*P. falciparum* n/N (%)	*P. vivax* n/N (%)	Mixed Infection n/N (%)
Nucleated red blood cells	32/74 (43.2)	18/39 (46.2)	11/27 (40.7)	3/8 (37.5)
Hypersegmented neutrophils	2/74 (2.7)	1/39 (2.6)	1/27 (3.7)	0/8 (0.0)
Toxic granulation	6/74 (8.1)	4/39 (10.3)	2/27 (7.4)	0/8 (0.0)
Atypical lymphocytes	4/74 (5.4)	3/39 (7.7)	1/27 (3.7)	0/8 (0.0)
Elevated reticulocyte count	31/74 (41.9)	17/39 (43.6)	11/27 (40.7)	3/8 (37.5)

Peripheral smear examination showed nucleated red blood cells in 43.2% of malaria-positive patients, indicating a frequent erythropoietic stress response. Elevated reticulocyte count was observed in 41.9% of patients. Toxic granulation and atypical lymphocytes were less frequent, occurring in 8.1% and 5.4% of cases, respectively. These findings were numerically more common in *P. falciparum* infection, although the available data do not support strong inference regarding species-specific differences for these smear findings.

Table 4. Comparative Analysis of Selected Hematological Parameters Between P. falciparum and P. vivax Mono-Infections

Parameter	*P. falciparum* n/N (%)	*P. vivax* n/N (%)	Reported p-value
Anemia	35/39 (89.7)	23/27 (85.2)	>0.05
Thrombocytopenia	31/39 (79.5)	16/27 (59.3)	>0.05
Relative lymphopenia	13/39 (33.3)	3/27 (11.1)	<0.04
Monocytosis	8/39 (20.5)	4/27 (14.8)	>0.05
Neutrophilia	6/39 (15.4)	2/27 (7.4)	>0.05
Atypical lymphocytes	3/39 (7.7)	1/27 (3.7)	>0.05

Direct comparison between *P. falciparum* and *P. vivax* mono-infections showed no statistically reported difference in anemia or thrombocytopenia.

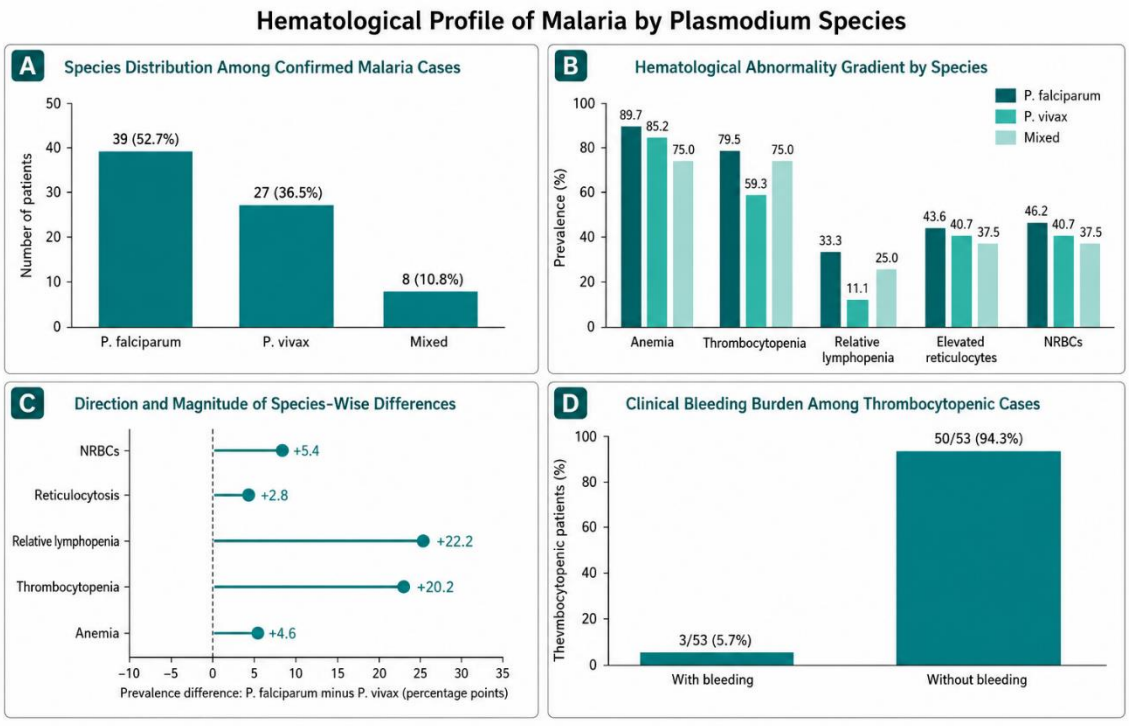

Figure 1 Among 74 confirmed malaria cases, P. falciparum accounted for 39 cases (52.7%), followed by P. vivax with 27 cases (36.5%) and mixed infection with 8 cases (10.8%). Anemia remained highly prevalent across species, affecting 89.7% of P. falciparum, 85.2% of P. vivax, and 75.0% of mixed infections. Thrombocytopenia showed a larger species-wise gradient, affecting 79.5% of P. falciparum cases compared with 59.3% of P. vivax cases, while relative lymphopenia showed the most pronounced proportional difference between mono-infections, with a +22.2 percentage-point excess in P. falciparum. Despite thrombocytopenia

in 53 patients, clinically significant bleeding was uncommon, occurring in only 3/53 thrombocytopenic cases (5.7%), supporting cautious interpretation of platelet count as a frequent hematological abnormality rather than a direct bleeding predictor.

Thrombocytopenia was numerically more frequent in *P. falciparum* infection, but the reported comparison did not reach statistical significance. Relative lymphopenia was the only reported parameter showing a statistically significant species-wise difference, occurring in 33.3% of *P. falciparum* cases compared with 11.1% of *P. vivax* cases. Other leukocyte and smear abnormalities, including monocytosis, neutrophilia, and atypical lymphocytes, did not show statistically reported interspecies differences.

Overall, confirmed malaria cases in this cohort demonstrated a high burden of hematological abnormalities, particularly anemia and thrombocytopenia. *P. falciparum* infection showed numerically higher frequencies of anemia, thrombocytopenia, relative lymphopenia, toxic granulation, atypical lymphocytes, and elevated reticulocyte count compared with *P. vivax*, but only relative lymphopenia was reported as statistically significant. These findings support the clinical value of routine complete blood count and peripheral smear assessment in malaria-positive patients, while the cross-sectional design and limited number of confirmed cases require cautious interpretation of species-wise differences.

DISCUSSION

The present study describes the hematological profile of confirmed malaria cases presenting to a tertiary-care hospital in Rawalpindi and demonstrates that anemia and thrombocytopenia were the dominant laboratory abnormalities among malaria-positive patients. Although *Plasmodium falciparum* was more frequent than *P. vivax* in this cohort, this finding should be interpreted in relation to the hospital-based sampling frame and local referral pattern rather than as a direct reflection of national malaria epidemiology. Previous Pakistani surveillance and regional studies have commonly reported *P. vivax* predominance in several endemic districts; therefore, the higher proportion of *P. falciparum* observed in the present sample may reflect geographic variation, tertiary-care case mix, military or referral population characteristics, or differences in the timing and setting of patient presentation (3,4).

Anemia was the most frequent hematological abnormality, affecting 86.5% of malaria-positive patients. The frequency was high in both *P. falciparum* and *P. vivax* mono-infections, suggesting that both species were associated with clinically important erythrocyte and marrow-related changes in this cohort. The mechanism of malaria-associated anemia is multifactorial and includes direct destruction of parasitized erythrocytes, immune-mediated clearance of non-parasitized erythrocytes, splenic sequestration, inflammatory suppression of erythropoiesis, and altered iron regulation (6,7). The comparable frequency of anemia across *P. falciparum* and *P. vivax* infections in the present study supports the need to avoid assuming that *P. vivax* infection is hematologically mild, particularly in settings where baseline anemia, nutritional vulnerability, delayed diagnosis, or repeated exposure may contribute to more pronounced blood-cell abnormalities.

Thrombocytopenia was the second most common abnormality and was observed in 71.6% of confirmed malaria cases. The frequency was numerically higher in *P. falciparum* infection than in *P. vivax* infection, although the reported comparison did not reach statistical significance. This pattern is consistent with previous regional observations showing that thrombocytopenia is a frequent laboratory feature of malaria and may occur across species (8,9). The biological basis of thrombocytopenia in malaria includes immune-mediated platelet destruction, splenic pooling, platelet activation, oxidative stress, endothelial interaction, and reduced platelet survival. Importantly, clinically significant bleeding occurred in only 5.7% of thrombocytopenic patients in the present study, indicating that platelet count alone should not be interpreted as a direct marker of hemorrhagic risk without clinical correlation. This finding supports the practical view that thrombocytopenia is a common supportive hematological clue in malaria but should be interpreted alongside bleeding manifestations, overall clinical status, and other laboratory indicators.

Relative lymphopenia was the only reported parameter showing a statistically significant difference between *P. falciparum* and *P. vivax* mono-infections. It was observed in 33.3% of *P. falciparum* cases compared with 11.1% of *P. vivax* cases. This finding may reflect greater immune-cell redistribution, inflammatory activation, or lymphocyte depletion associated with *P. falciparum* infection, as described in studies evaluating hematological and inflammatory biomarkers in falciparum malaria (10). However, the present study measured relative lymphocyte percentage rather than absolute lymphocyte count, and clinical severity outcomes were not formally analyzed. Therefore, lymphopenia should be interpreted as a species-associated hematological difference in this cohort rather than as a validated severity or prognostic biomarker. Future studies should evaluate absolute lymphocyte count, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, parasite density, and clinical severity categories in an adjusted analytical model (11).

Peripheral smear findings further demonstrated the hematological stress associated with malaria infection. Nucleated red blood cells were present in 43.2% of malaria-positive patients, and elevated reticulocyte count was observed in 41.9%, suggesting compensatory erythropoietic activity in response to hemolysis and red-cell destruction. Toxic granulation and atypical lymphocytes were less frequent but reflected inflammatory and immune-cell activation. These smear-based findings are clinically relevant because peripheral smear examination remains an accessible diagnostic and interpretive tool in many resource-limited settings. However, because the study was cross-sectional, these findings cannot determine whether morphological abnormalities predict recovery time, complications, or treatment response.

The study has several limitations. First, the single-center tertiary-care design limits generalizability to primary-care, rural, and community settings where malaria epidemiology and disease severity may differ. Second, only 74 of 400 clinically suspected patients were malaria-positive, resulting in relatively small species-specific subgroups and limited statistical power to detect modest differences between *P. falciparum* and *P. vivax*. Third, the analysis was primarily descriptive and bivariate, without adjustment for potential confounders such as age, sex, nutritional status, fever duration, baseline anemia, prior antimalarial exposure, comorbid infection, or parasite density. Fourth, relative lymphocyte percentages were used rather than absolute lymphocyte counts, limiting interpretation of true lymphopenia. Fifth, although parasite density calculation was described in the methodology, parasite-density results were not presented, preventing analysis of hematological abnormalities by parasitemia burden. Finally, diagnostic classification based on microscopy and/or rapid diagnostic testing may introduce misclassification if discordant results are not resolved through a prespecified reference approach.

Despite these limitations, the study provides useful local evidence that malaria-positive patients in this Pakistani tertiary-care setting commonly present with anemia, thrombocytopenia, and smear evidence of erythropoietic stress. The findings emphasize the clinical value of routine complete blood count and peripheral smear review in suspected or confirmed malaria. However, the results should be interpreted as descriptive and species-associated rather than causal, diagnostic, or prognostic. Multicenter prospective studies with standardized diagnostic adjudication, parasite-density reporting, absolute leukocyte differentials, clinical severity grading, and adjusted statistical analysis are needed to validate whether routine hematological parameters can meaningfully support risk stratification in malaria management.

CONCLUSION

Malaria-positive patients in this tertiary-care cohort demonstrated a high frequency of hematological abnormalities, particularly anemia and thrombocytopenia. Both *P. falciparum* and *P. vivax* infections were associated with substantial hematological involvement, while relative lymphopenia was more frequently observed in *P. falciparum* mono-infection. Clinically significant bleeding was uncommon despite the high prevalence of thrombocytopenia, indicating that platelet count should be interpreted in

clinical context rather than as an isolated predictor of bleeding. Routine complete blood count and peripheral smear assessment remain valuable supportive tools in the evaluation of malaria patients, but the cross-sectional design and limited subgroup size require cautious interpretation. Further multicenter prospective studies are needed to evaluate absolute blood-cell parameters, parasite density, clinical severity, and adjusted predictors of hematological outcomes in malaria.

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