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Haematological Profiles of Malarial Patients with and Without Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency in Mardan, Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Malaria, caused by protozoan parasites of the genus Plasmodium (family Plasmodiidae, phylum Apicomplexa), remains a significant global health challenge. This study investigated the haematological profiles and prevalence of glucose-6-phosphate dehydrogenase (G6PD) deficiency in malaria patients infected with Plasmodium vivax in Mardan, Pakistan. Blood samples were collected from 104 malaria-positive patients seeking treatment at local hospitals, with informed written consent obtained from all participants or their guardians (for minors). Malaria was diagnosed via standard microscopic examination of Giemsa-stained blood films, and all samples were tested for G6PD deficiency. Haematological parameters, including hemoglobin, hematocrit, red blood cells (RBCs), white blood cells (WBCs), platelets, neutrophils, eosinophils, monocytes, lymphocytes, mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC), were assessed using a complete blood count (CBC) performed on a hematology analyzer. Data were analyzed using Pearson correlation and Chi-square tests to evaluate associations between malaria infection, haematological parameters, and G6PD activity. Of the 104 cases, 56 (53.8%) were male and 48 (46.2%) were female, with G6PD deficiency identified in 4 (3.8%) patients (3 males, 1 female). Haematological abnormalities included anemia (65%), thrombocytopenia (59.6%), leukopenia or leukocytosis (30.8%), and variations in MCV and MCH. Significant positive correlations were observed between hemoglobin and hematocrit, MCHC and hemoglobin, and lymphocytes and WBCs, while negative correlations were noted between RBCs and hematocrit, eosinophils and RBCs, and eosinophils and MCV. In G6PD-deficient patients, hemoglobin, WBCs, RBCs, platelets, MCV, and lymphocytes were consistently affected, with monocytes, eosinophils, and hematocrit altered in some cases. No significant correlations were found for age, platelets, hematocrit, neutrophils, WBCs, RBCs, MCV, MCHC, or lymphocytes among malaria patients, but hemoglobin showed significant variation in relation to G6PD deficiency. These findings confirm the prevalence of P. vivax malaria in Mardan and highlight its impact on haematological parameters, particularly in G6PD-deficient individuals. Further studies are warranted to explore the therapeutic implications of the association between G6PD deficiency and altered hemoglobin levels in malaria.

INTRODUCTION

Malaria is a severe infectious disease caused by *Plasmodium* parasites, which are transmitted via the bites of female *Anopheles* mosquitoes, and continues to pose a major global health challenge (Kamau, 2020). In 2022, the Global Health Organization estimated 249 million malaria cases and 608,000 deaths globally, with sub-Saharan Africa representing 80% of cases, predominantly affecting children under five (Umugwaneza et al., 2025). Global efforts to reduce malaria incidence have encountered stagnation, evidenced by a 5 million case increase from 2021 to 2022. This rise is attributed to factors including climate change, drug resistance, and disruptions in healthcare delivery (Benavente et al., 2021). Malaria affects regions beyond Africa, with Southeast Asia, particularly Pakistan, playing a substantial role in the global incidence of *Plasmodium vivax* infections (Mubaraki et al., 2024).

Malaria is endemic in Pakistan, with approximately 3.4 million suspected cases reported in 2022, of which 1.7 million were confirmed (Howes et al., 2016). *Plasmodium vivax* is the predominant species, accounting for 67% of cases, while *Plasmodium falciparum* represents 32%, and mixed infections comprise 1% (Campo et al., 2015).

Plasmodium vivax presents distinct challenges owing to its capacity to develop dormant liver-stage hypnozoites, which may lead to relapses months or years post-initial infection, thereby complicating treatment and control strategies (Naser et al., 2024). The epidemiology of malaria in Pakistan is regionally diverse, shaped by climate, urbanization, and socioeconomic conditions, with seasonal peaks occurring after monsoon floods (Pang et al., 2025). Malaria incidence in Khyber Pakhtunkhwa, particularly in Mardan, is elevated due to conducive conditions for mosquito breeding and restricted access to healthcare services (Mubaraki et al., 2024).

Malaria infections are often linked to hematological abnormalities that significantly impact morbidity and mortality rates. Common complications encompass anemia, thrombocytopenia, leukopenia, leukocytosis, and, less frequently, disseminated intravascular coagulation (Fernandes, 2013; Naser et al., 2024). These alterations arise from hemolysis induced by parasites, immune-mediated destruction of erythrocytes, and suppression of bone marrow (Qidwai, 2021). *P. falciparum* infections are well-documented for inducing severe malaria anemia, characterized by hemoglobin levels falling below 5 g/dL in children or 7 g/dL in adults, with prevalence rates between 30% and 90% in endemic regions (Nguyen et al., 2025). *P. vivax* infections, although typically less severe, frequently lead to thrombocytopenia and mild-to-moderate anemia, especially in non-immune individuals (Taleb et al., 2025). The mechanisms underlying these abnormalities differ according to parasite species, host immunity, nutritional status, and coexisting conditions, highlighting the importance of region-specific studies for comprehending local disease patterns (Ali et al., 2005).

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-linked genetic disorder with significant prevalence in malaria-endemic regions, impacting more than 400 million individuals worldwide (Paika et al., 2025). The prevalence of G6PD deficiency in Pakistan varies between 2% and 10%, with elevated rates observed in specific ethnic groups (Bancone & Chu, 2021). The enzyme G6PD serves a protective role for red blood cells against oxidative stress, with its deficiency potentially resulting in hemolytic anemia, especially when induced by medications such as primaquine, which is essential for the eradication of *P. vivax* hypnozoites (Shehzad et al., 2025). G6PD deficiency offers partial protection against malaria by restricting parasite survival in red blood cells; however, it complicates treatment, as primaquine can induce severe hemolysis in affected individuals (Ali et al., 2024). It is advisable to test for G6PD deficiency prior to administering primaquine; however, this testing is frequently inaccessible in resource-limited environments such as Pakistan, thereby heightening the risk of negative consequences (Lowe et al., 2008).

Although malaria poses a significant burden in Pakistan, there is a lack of comprehensive studies examining the relationship between *P. vivax* infection, hematological profiles, and G6PD deficiency (Ali et al., 2005). Current research predominantly centers on *P. falciparum* and lacks comprehensive analyses of G6PD status concerning hematological alterations. This gap is especially significant in areas such as Mardan, where *P. vivax* is the predominant species and G6PD deficiency is prevalent. Comprehending these interactions is essential for enhancing treatment strategies, reducing complications, and guiding malaria control initiatives. This research examines the haematological profiles of *P. vivax*-infected patients in Mardan, Pakistan, contrasting individuals with G6PD deficiency against those without. The study aims to clarify the influence of G6PD status on hematological parameters, thereby offering insights into safer and more effective therapeutic strategies for malaria management in endemic regions.

This study is the first to comprehensively examine the links between P. vivax malaria, hematological changes, and G6PD deficiency in Mardan, Pakistan. Unlike prior focus on P. falciparum, it contrasts profiles in G6PD-deficient vs. non-deficient patients, providing novel insights for safer treatments and control in resource-poor areas.

MATERIAL AND METHODS

Study Design

This cross-sectional study was conducted in Mardan, Khyber Pakhtunkhwa, Pakistan, from June to October 2023. Mardan, the second-largest city in the province, spans approximately 32 km² and is located at 72°3′11″–72°14′48″E longitude and 34°9′4″–34°13′21″N latitude. Known for its agricultural sector and historical landmarks, Mardan experiences a high malaria burden, particularly Plasmodium vivax infections, due to favorable climatic conditions.

Figure 1

Study Area Mardan, Khyber Pakhtunkhwa, Pakistan

Study Population and Sampling

A purposive sampling strategy was employed to recruit 104 patients diagnosed with P. vivax malaria at hospitals and medical centers in Mardan and the samples were collected in june/octobar 2023. Inclusion criteria included confirmed P. vivax infection, willingness to participate, and provision of informed consent. Both male and female patients of all ages were included. Exclusion criteria encompassed co-infections, chronic illnesses, or refusal to consent. Demographic and clinical data were collected using a pre-structured questionnaire, capturing age, sex, symptoms, and medical history.

Sample Collection

Venous blood samples (3 mL) were collected via venipuncture into EDTA tubes following standard phlebotomy protocols. Materials used included safety

needles, butterfly needles (21-gauge or smaller), Vacutainer tube holders, blood collection tubes with color-coded additives, single-use latex-free tourniquets, 70% isopropyl alcohol wipes, 2×2 gauze, and adhesive bandages. Sharps were disposed of in puncture-proof, OSHA-compliant biohazard containers. Whole blood samples were aliquoted for haematological and parasitological analyses and stored at 4°C until processing (Ali et al., 2024).

Laboratory Procedures

Malaria Diagnosis: Malaria was diagnosed using standard microscopic examination of Giemsa-stained peripheral blood smears, considered the gold standard for P. vivax detection. Thick and thin blood films were prepared from finger-prick blood. Thin films were fixed with 100% methanol, and both thick and thin films were stained with 10% Giemsa for 10 minutes. Slides were examined by experienced microscopists under a 100× oil immersion objective to confirm P. vivax parasitemia.

G6PD Deficiency Testing: Glucose-6-phosphate dehydrogenase (G6PD) activity was quantitatively assessed using the RANDOX G6PD test kit (RANDOX Laboratories, UK), following the manufacturer's instructions. Results were recorded in units per gram of hemoglobin (U/g Hb) to classify participants as G6PD-deficient or normal.

Complete Blood Count (CBC): Haematological profiles were determined using an automated hematology analyzer Sysmex XN-1000. The CBC included hemoglobin, hematocrit, red blood cell (RBC) count, white blood cell (WBC) count, differential counts (neutrophils, lymphocytes, monocytes, eosinophils, basophils), platelet count, and RBC indices (mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC)). Analyses adhered to the manufacturer's protocols and industry standards, with results printed for data entry.

Data Management: Data on demographic characteristics, clinical history, G6PD activity, and haematological parameters were entered into Microsoft Excel 2013 for organization and preliminary analysis. Each participant's data was anonymized and coded to ensure confidentiality.

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS Version 21) Descriptive statistics, including means, frequencies, and percentages, were calculated for demographic and haematological parameters. Pearson's correlation coefficient was used to assess associations between haematological parameters and their significance. The Chi-square test evaluated associations between G6PD status and haematological profiles. A p-value <0.05 was considered statistically significant for all tests.

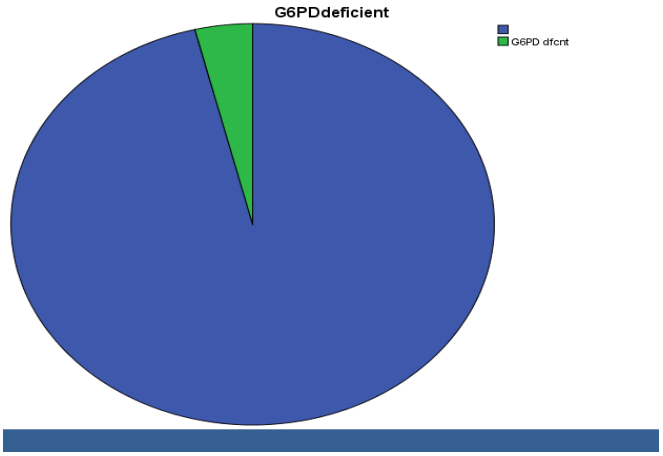
RESULTS

Study Population and G6PD Deficiency

This cross-sectional study, conducted from June to October 2023 across multiple hospitals in Mardan, Khyber Pakhtunkhwa, Pakistan, enrolled 104 patients with confirmed Plasmodium vivax malaria, diagnosed via peripheral blood smear microscopy and rapid diagnostic tests (RDTs). The study population included 56 males (53.8%) and 48 females (46.2%). Glucose-6-phosphate dehydrogenase (G6PD) deficiency was identified in 4 patients (3.8%), comprising 3 males (2.9%) and 1 female (1.0%). The remaining 100 patients (96.2%) exhibited normal G6PD activity. The distribution of G6PD deficiency among the study population is illustrated in Figure 1.

Figure 1

Pie chart showing the distribution of G6PD deficiency among Plasmodium vivax-infected patients in Mardan, Pakistan (n=104). Blue represents G6PD-sufficient patients (96.2%), and green represents G6PD-deficient patients (3.8%).

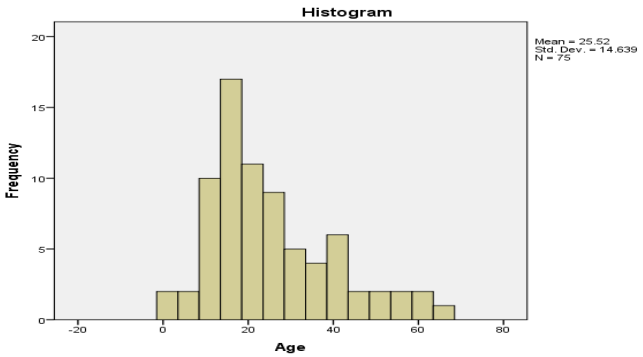


Age Distribution of Study Participants

Participant ages ranged from 1 to 65 years, with a mean age of 25.5 years (SD = 14.6). The most frequently observed age was 18 years, indicating a higher prevalence of Plasmodium vivax infection among younger adults in the study population. The age distribution is illustrated in Figure 2, which shows that the majority of cases clustered in the younger age groups, with fewer cases observed among older participants.

Figure 2

Histogram illustrating the age distribution of Plasmodium vivax-infected patients in Mardan, Pakistan (n = 75). Ages ranged from 1 to 65 years, with the most frequent age being 18 years.



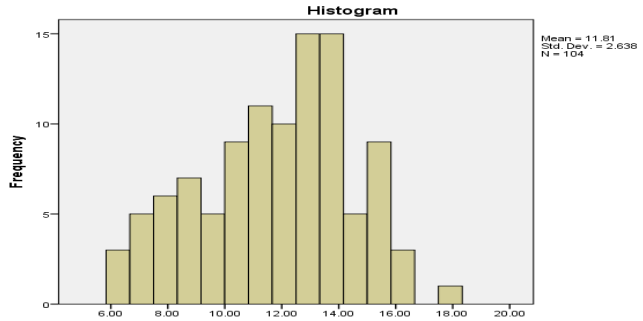
Hematological Findings in P. Vivax-Infected Patients

Among the 104 patients with Plasmodium vivax infection, a range of hematological abnormalities were observed, including anemia, thrombocytopenia, leukopenia, leukocytosis, and variations in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values. Specifically, hemoglobin levels were affected in 68 patients, platelet counts in 62, hematocrit in 12, white

blood cells (WBCs) in 32, red blood cells (RBCs) in 35, monocytes in 32, neutrophils in 49, and eosinophils in 26 cases. Among the patients with abnormal hemoglobin levels, 41 (60.2%) were male and 27 (39.7%) were female. Hemoglobin values ranged from a minimum of 5.90 g/dl to a maximum of 18.00 g/dl, with a difference of 12.10 g/dl, as illustrated in Figure 3.

Figure 3

Histogram showing the distribution of hemoglobin levels among P. vivax-infected patients in Mardan, Pakistan (n=104). The mean hemoglobin was 11.81 g/dl (SD = 2.64), with values ranging from 5.90 to 18.00 g/dl.

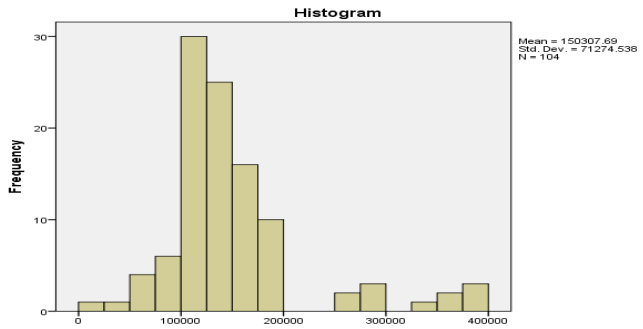


Platelet Count Distribution

The distribution of platelet counts among the 104 Plasmodium vivax-infected patients is shown in Figure 4. Platelet values ranged from a minimum of 11,000/µL to a maximum of 392,000/µL, with the highest frequency observed at 120,000/µL. The mean platelet count was 150,307/µL (SD = 71,274), indicating that thrombocytopenia was common in the study population. The histogram demonstrates that the majority of patients had platelet counts clustered between 100,000/µL and 200,000/µL, while only a few cases exhibited extremely low or high platelet counts. These findings highlight the significant variation in platelet levels among P. vivax-infected individuals, with most patients experiencing moderate to severe reductions in platelet count.

Figure 4

Histogram illustrating the distribution of platelet counts among Plasmodium vivax-infected patients in Mardan, Pakistan (n = 104). The most frequently observed platelet count was 120,000/µL, with values ranging from 11,000/µL to 392,000/µL.

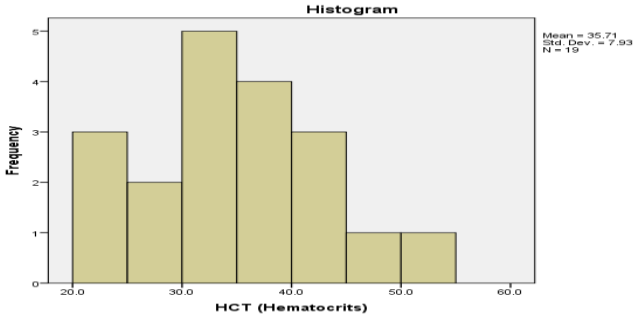


Hematocrit Distribution

Among the 12 patients identified with hematocrit deficiency, the gender distribution was equal, with 6 males (50%) and 6 females (50%). The hematocrit values ranged from 20.9% to 50.1%, with a mean of 35.71% and a standard deviation of 7.93%, indicating moderate variability within the group. The distribution is slightly right-skewed, as shown in Figure 5, with the majority of patients exhibiting hematocrit values clustered between 30% and 40%. This pattern suggests that while most patients had moderate reductions in hematocrit, a small number experienced more severe anemia, as reflected by the lower tail of the distribution. The relatively wide range and standard deviation highlight the heterogeneity in hematocrit response among Plasmodium vivax-infected individuals.

Figure 5

Histogram illustrating the distribution of hematocrit values among malarial patients in Mardan, Pakistan (n = 19). The mean hematocrit was 35.71% (SD = 7.93), with values ranging from 20.9% to 50.1%.

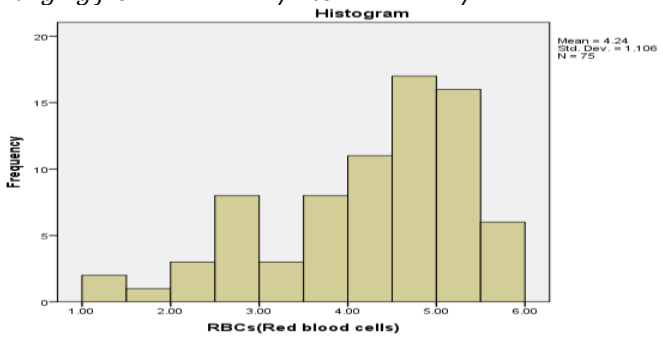


Red Blood Cell (RBC) Count Distribution

Abnormal red blood cell (RBC) counts were observed in 35 malaria patients, comprising 17 males (48.5%) and 18 females (51.4%). The RBC counts in the study population ranged from a minimum of 1.12×10^{12}/L to a maximum of 5.90×10^{12}/L, with a total variation (range) of 4.78×10^{12}/L. The mean RBC count was 4.24×10^{12}/L (SD = 1.06), as indicated in Figure 6. The histogram demonstrates a right-skewed distribution, with the highest frequency of cases observed at 2.91×10^{12}/L, suggesting that a considerable proportion of patients experienced moderate to severe reductions in RBC count. These findings highlight significant variability in erythrocyte levels among Plasmodium vivax-infected individuals, reflecting the hematological impact of malaria in the study region.

Figure 6

Histogram illustrating the distribution of RBC counts among malarial patients in Mardan, Pakistan (n = 75). The mean RBC count was 4.24 × 10^{12}/L (SD = 1.06), with values ranging from 1.12 × 10^{12}/L to 5.90 × 10^{12}/L.



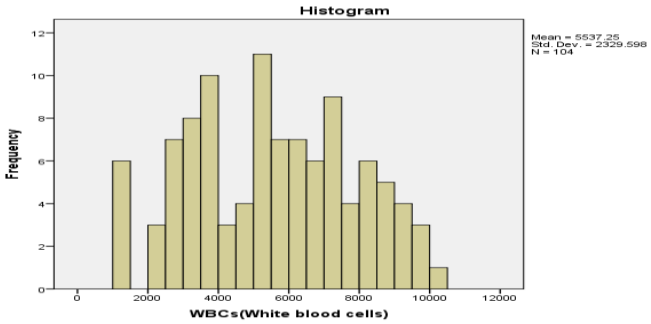
White Blood Cell (WBC) Count Distribution

In the cohort of 104 Plasmodium vivax-infected patients, abnormal white blood cell (WBC) counts were observed

in 32 individuals, with 18 males (56.2%) and 14 females (43.7%) affected. The majority of patients (72 cases, 73.8%) exhibited WBC counts within the normal reference range. The WBC counts ranged from a minimum of 1,120/µL to a maximum of 10,000/µL, with a mean value of 5,537/µL (SD = 2,329.6), as shown in Figure 7. The histogram reveals a moderately right-skewed distribution, with the highest frequency observed at 7,400/µL. This distribution indicates substantial variability in WBC counts among the study population, with most cases clustering between 4,000/µL and 8,000/µL. The observed range and standard deviation reflect notable heterogeneity in the immunological response to malaria infection within this population.

Figure 7

Histogram illustrating the distribution of white blood cell (WBC) counts among malarial patients in Mardan, Pakistan (n = 104). The mean WBC count was 5,537/µL (SD = 2,329.6), with values ranging from 1,120/µL to 10,000/µL and the most frequent value at 7,400/µL

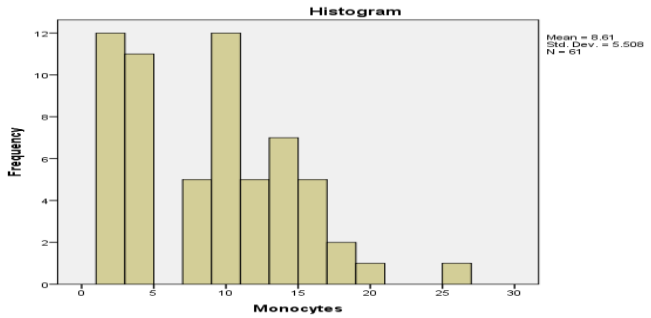


Monocyte Count Distribution

Among 61 malarial patients from Mardan, abnormal monocyte counts were observed in 32 individuals (30%), with an equal gender distribution (16 males and 16 females). The monocyte counts ranged from a minimum of 2 to a maximum of 25, with a mean value of 8.61 (SD = 5.508). The histogram (Figure 8) demonstrates a right-skewed distribution, with the highest frequencies observed in the lower monocyte ranges (2–5 and 10–12). Most cases clustered between 2 and 15 monocytes, indicating substantial variability in monocyte counts among the study population. The observed range and standard deviation reflect notable heterogeneity in the immunological response to malaria infection within this group.

Figure 8

Histogram illustrating the distribution of monocyte counts among malarial patients in Mardan, Pakistan (n = 61). The mean monocyte count was 8.61 (SD = 5.508), with values ranging from 2 to 25.

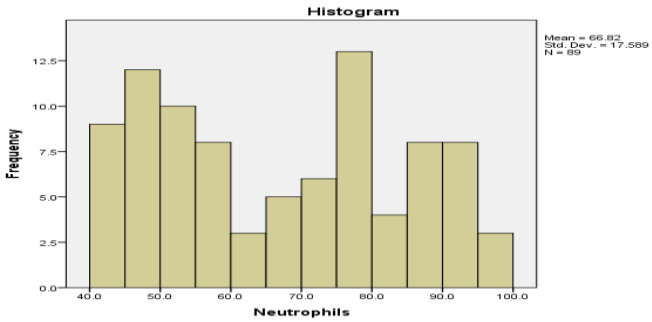


Neutrophil Count Distribution in Malarial Patients

In a cohort of malarial patients, 25 males (51.0%) and 24 females (48.9%) exhibited abnormal neutrophil levels. The neutrophil counts ranged from a minimum of 40 to a maximum of 99, with a variation of 59. The most frequently observed neutrophil count was 49. This distribution suggests considerable heterogeneity in neutrophil response among the infected individuals, which aligns with previous studies indicating that neutrophil levels can vary widely during malaria infection depending on factors such as disease severity and parasitemia levels (Figure 9).

Figure 9

Histogram illustrating the distribution of neutrophil counts among malarial patients in Mardan, Pakistan (n = 49). The neutrophil counts ranged from 40 to 99, with the most frequent value at 49 and a variation of 59. The distribution shows a broad spread of neutrophil levels, reflecting the diverse immunological responses in the study population.

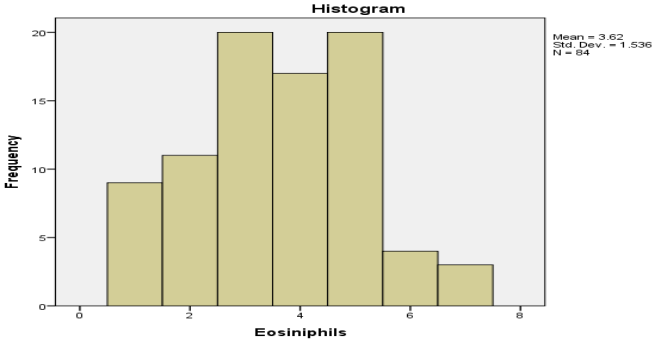


Eosinophil Count Distribution in Malarial Patients

In this study of malaria cases, 26 patients (27.5%) exhibited abnormal eosinophil levels, including 10 males (38.4%) and 16 females (61.5%). The eosinophil counts ranged from a minimum of 1 to a maximum of 7, with a variation of 6. The most frequently observed eosinophil count was 3. This distribution suggests a mild eosinophilic response in a subset of malarial patients, consistent with findings that eosinophil levels can vary during malaria infection and may be influenced by factors such as infection stage and coexisting parasitic conditions (Figure 10).

Figure 10

Histogram illustrating the distribution of eosinophil counts among malarial patients in Mardan, Pakistan (n = 95). The eosinophil counts ranged from 1 to 7, with the most frequent value at 3 and a variation of 6. The histogram shows that most patients had eosinophil counts clustered around the lower range, reflecting the typical mild eosinophilic response seen in malaria infection.

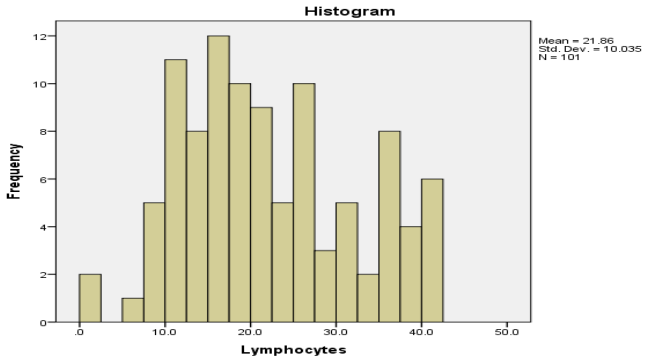


Lymphocyte Count Distribution in Malarial Patients

Among the malaria patients surveyed, 49 individuals (48.5%) exhibited abnormal lymphocyte levels, comprising 25 males (51.0%) and 24 females (48.9%). The lymphocyte counts ranged from a minimum of 1 to a maximum of 40, with a variation of 39 observed across cases. The most frequently occurring lymphocyte count was 13. This distribution reflects significant heterogeneity in lymphocyte response among malarial patients, consistent with prior studies showing that malaria infection can lead to lymphopenia or altered lymphocyte profiles due to immune system activation and redistribution (Figure 11).

Figure 11

Histogram illustrating the distribution of lymphocyte counts among malarial patients in Mardan, Pakistan (n = 101). The lymphocyte counts ranged from 1 to 40, with the most frequent value at 13 and a variation of 39. The distribution shows a wide range of lymphocyte counts, indicating diverse immunological responses to malaria infection within the study population.



Association among Different Haematological Profiles

Pearson correlation coefficients among haematological parameters in malaria patients ($P < 0.05$). Significant positive correlations were identified between hemoglobin (Hb) and hematocrit (HCT) (r = 0.458), mean corpuscular hemoglobin concentration (MCHC) and Hb (r = 0.131), and lymphocytes and white blood cells (WBCs) (r = 0.210), indicating synchronized alterations in red cell indices and immune cell counts. Significant negative correlations were observed between red blood cells (RBCs) and HCT (r = -0.179), eosinophils and RBCs (r = -0.201), and eosinophils and mean corpuscular volume (MCV) (r = -0.077), suggesting inflammatory or parasitic effects on red cell parameters. Among G6PD-deficient patients (n = 4), all showed abnormalities in Hb, RBCs, MCV, platelets, and lymphocytes, with WBCs affected in all cases, HCT in one, and monocytes and eosinophils in two, reflecting pronounced haematological impacts. These findings highlight the intricate relationships among haematological profiles in malaria, offering insights into disease pathophysiology and clinical management.

Mann-Whitney U Test

The Mann-Whitney U test was utilized to evaluate differences in haematological parameters between malaria patients with and without G6PD deficiency, with significance set at $P < 0.05$ (Table 2). No significant differences were observed in age, platelets, hematocrit, neutrophils, white blood cells, red blood cells, mean corpuscular volume, mean corpuscular hemoglobin concentration, or lymphocytes ($P > 0.05$). However, a significant difference was found in hemoglobin levels ($P = 0.024$), indicating that G6PD deficiency is associated with altered hemoglobin in malaria patients, potentially exacerbating anemia. These findings suggest that G6PD deficiency primarily impacts hemoglobin among haematological parameters, highlighting its role in malaria-related hematological complications and underscoring the need for targeted clinical monitoring in affected individuals.

Table 1

Pearson Correlation Coefficients among Haematological Parameters in Malaria Patients (P < 0.05)

	Age	Hb	PLT	HCT	Neut	Mono	WBC	RBC	MCV	MCHC	Lymph	Eos
Age	1.000	-0.147	0.205	-0.067	-0.066	0.178	-0.137	-0.153	-0.026	-0.053	0.063	0.406
Hb		1.000	-0.181	0.458*	-0.045	-0.113	0.180	0.390	0.196	0.131*	-0.140	-0.010
PLT			1.000	-0.003	-0.007	0.057	-0.108	-0.207	-0.058	-0.128	0.097	0.130
HCT				1.000	-0.303	-0.384	-0.412	-0.179*	0.189	0.315	-0.478	-0.574
Neutrophils					1.000	0.170	0.239	0.004	-0.161	-0.050	0.155	0.070
Monocytes						1.000	0.083	-0.520	-0.466	-0.262	0.163	0.272
WBCs							1.000	0.303	0.044	0.049	0.210*	-0.029
RBCs								1.000	0.265	0.155	-0.016	-0.201*
MCV									1.000	0.304	-0.214	-0.077*
MCHC										1.000	-0.084	-0.006
Lymphocytes											1.000	0.163
Eosinophils												1.000

Table 2

Mann-Whitney U Test Results for Haematological Parameters in Malaria Patients with and without G6PD Deficiency

Variable	N	Minimum	Maximum	Mean	SD	P-value
Age (years)	75	1.0	65.0	25.52	14.639	0.159
Hemoglobin (g/dL)	104	5.90	18.00	11.81	2.637	0.024*
Platelets (/µL)	104	11,000	392,000	150,307.69	71,274.54	0.775
Hematocrit (%)	19	20.9	50.1	35.71	7.930	0.316
Neutrophils (%)	89	40.0	99.0	66.82	17.589	0.718
WBCs (/µL)	104	1,120	10,000	5,537.25	2,329.60	0.666
RBCs ($\times 10^6$/µL)	75	1.12	5.90	4.24	1.106	0.103
MCV (fL)	75	31.2	98.3	76.92	15.077	0.501
MCHC (g/dL)	75	7.90	42.00	31.67	4.329	0.458
Lymphocytes (%)	101	1.0	40.0	21.86	10.036	0.077

Multivariate Analysis

Multivariate analysis was performed to investigate the relationship between G6PD deficiency status and factors including age and haematological parameters in malaria patients. The analysis revealed no significant associations ($P > 0.05$), indicating that neither age nor haematological parameters significantly predict G6PD deficiency in this cohort. This lack of association may reflect the influence of unmeasured confounders or the limited sample size, suggesting the need for further research to elucidate the factors driving G6PD deficiency in malaria.

Chi-Square Test

The Chi-Square test was employed to examine associations between G6PD deficiency and categorical variables in malaria patients, with significance set at $P < 0.05$ (Table 3). No significant associations were observed between G6PD status and gender ($P = 0.387$), monocytes ($P = 0.057$), or mean corpuscular hemoglobin (MCH) ($P = 0.835$). These findings suggest that G6PD deficiency is not significantly linked to gender or the tested haematological parameters, indicating that other biological or environmental factors may play a more prominent role in G6PD-related effects in malaria patients.

Table 3

Chi-Square Test Results for Association of Haematological Parameters and Gender with G6PD Deficiency

Variable	P-value
Gender	0.387
Monocytes	0.057
Mean Corpuscular Hemoglobin (MCH)	0.835

DISCUSSION

Malaria, a major public health challenge in tropical and subtropical regions, affects approximately 40% of the global population, predominantly in low-income countries, with sub-Saharan Africa bearing the highest burden (Khan et al., 2023). In Pakistan, a tropical nation with extensive irrigation and standing water post-rainfall, mosquito breeding thrives, leading to year-round malaria prevalence, peaking from July to November (Bousema & Baidjoe, 2013). Regional variations in malaria distribution are influenced by local factors such as mosquito breeding conditions, health education, and genetic predispositions (Ali et al., 2024). This study investigates haematological parameters in malaria patients with and without glucose-6-phosphate dehydrogenase (G6PD) deficiency in Mardan, Pakistan, focusing on their association with G6PD status.

Among 104 malaria patients, 4 (3.84%) were diagnosed with G6PD deficiency, consistent with prior studies reporting low G6PD prevalence in malaria-positive individuals. All infections were caused by *Plasmodium vivax*, aligning with its high prevalence in Pakistan (98% in Okra, 90.4% in Muzaffarabad) compared to *P. falciparum*. Patients ranged in age from 1 to 65 years (mean: 25.52), with most being 18 years old, and males were more affected than females in both G6PD-deficient and non-deficient groups. However, no significant gender association with G6PD deficiency was found ($P = 0.387$, Chi-Square test), supporting prior findings.

Of the 104 patients, 68 were anemic, a common malaria complication driven by hemolysis, impaired erythropoiesis, and splenic sequestration. All four G6PD-deficient patients were anemic, and hemoglobin showed a significant difference associated with G6PD status ($P = 0.024$, Mann-Whitney U test) and positive correlations with hematocrit ($r = 0.458$, $P < 0.05$) and red blood cells (RBCs) ($r = 0.390$, $P < 0.05$). Other parameters (platelets, hematocrit, WBCs, RBCs, neutrophils, lymphocytes, eosinophils, MCHC, MCH) showed no significant differences with G6PD status ($P > 0.05$). Recent studies suggest G6PD deficiency may not directly impact red cell distribution width (RDW) in malaria but may influence monocyte counts (Khan & Ali, 2025).

G6PD activity, which peaks in reticulocytes and declines with erythrocyte aging, increases during acute malaria, potentially reducing the risk of drug-induced hemolysis during treatment (Paika et al., 2025) . This dynamic G6PD activity underscores the need for careful antimalarial drug selection, as certain medications (e.g., 8-aminoquinolines) can exacerbate hemolysis in G6PD-deficient patients (Bancone & Chu, 2021; Sadhewa et al., 2023). Early identification of G6PD deficiency is critical for optimizing treatment and improving outcomes in malaria management.

CONCLUSION

This study in Mardan, Pakistan, revealed a low prevalence of G6PD deficiency (3.84%) among 104 malaria patients, all infected with Plasmodium vivax. Anemia was prevalent in 68 patients, with all four G6PD-deficient individuals affected, and hemoglobin levels showed a significant association with G6PD status ($P = 0.024$). Significant correlations were observed between hemoglobin and hematocrit ($r = 0.458$) and red blood cells ($r = 0.390$), but other haematological parameters (platelets, WBCs, neutrophils, lymphocytes, eosinophils, MCHC, MCH) showed no significant association with G6PD deficiency. The absence of gender-specific trends and the predominance of P. vivax infections align with regional malaria patterns. These findings underscore the critical role of hemoglobin alterations in G6PD-deficient malaria patients and highlight the need for targeted screening to optimize treatment and mitigate complications such as drug-induced hemolysis.

Recommendations

- Routinely test for G6PD deficiency in malaria patients to guide safe antimalarial therapy.
- Monitor and treat anemia, especially in G6PD-deficient patients, with targeted interventions.
- Conduct prospective studies with larger cohorts and confirmatory G6PD testing to assess prevalence and impacts.
- Include malaria-free controls in future research to compare haematological changes.
- Enhance mosquito control and health education to reduce P. vivax transmission.

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