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Original Article

Effects of Malarial Parasitaemia on Haematological Parameters and Gender Distribution in District Gamba, Gilgit-Baltistan

Mustajab Alam, Usama Bin Khalid*, Ali Ahmed, Qasim Zia***, Ali Ghawas**

Department of Pathology, Combined Military Hospital, Skardu/National University of Medical Sciences (NUMS) Pakistan, *Department of Pathology, Combined Military Hospital, Dera Nawab Sahib/National University of Medical Sciences (NUMS) Pakistan, **Department of Pathology, Combined Military Hospital, Malir/ National University of Medical Sciences (NUMS) Pakistan, ***Department of Medicine, 123 Medical Battalion, Skardu Pakistan

ABSTRACT

Objective: To determine the effects of malaria infection on haematological parameters, and to know common parasite species of malaria in Skardu region (Gilgit-Baltistan).

Study Design: Cross-sectional study.

Place and Duration of Study: Combined Military Hospital, Skardu Pakistan, from Jan to Dec 2022.

Methodology: A group of 100 patients with various malaria symptoms, all of whom had tested positive for the disease, was enrolled. Written consent was obtained before enrolment, and their confidentiality was ensured at all levels. Blood smears were examined to identify and quantify malaria parasites, and a Sysmex XP 300 haematology analyser was used to measure haematological parameters. Several haematological parameters were studied in the infected participants, including WBCs, RBCs, platelets, Hb, haematocrit, MCV, MCH, neutrophil, and lymphocyte percentages. Descriptive statistics were presented as Mean±SD.

Results: Out of total patients, 64% patients were male and 36% were female. Out of the total cases of malaria infection, 80% were diagnosed with Plasmodium vivax, 12% with Plasmodium falciparum, and 8% had a mixed infection. A stronger connection between Plasmodium vivax and thrombocytopenia, with a *p*-value of 0.020, was observed, whereas Plasmodium falciparum was more strongly associated with anaemia and leukopenia.

Conclusions: Participants enrolled in the study who contracted malaria exhibited significant alterations in several blood-related characteristics. Among these, anaemia, thrombocytopenia, lymphopenia, monocytosis, and eosinopenia emerged as the key indicators of malaria infection, particularly in cases involving the Plasmodium falciparum species.

Keywords: Anaemia, Haematological Parameters, Malaria, Thrombocytopenia.

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INTRODUCTION

Human health and quality of life continue to be affected negatively by malaria, the world's most important protozoan infection. Despite the decrease in malaria mortality over the last decade, the most recent World Malaria Report revealed 228 million clinical cases, resulting in no fewer than 405,000 deaths.[1] Malaria ranks as the fifth-most prevalent cause of mortality on a global scale. The World Health Organization (WHO) estimates that 40% of the world's population is susceptible to malaria.[2] Environmental factors within a region and the socioeconomic status of the population are influential in the epidemiology of malaria.[3] Pakistan has a high prevalence of malaria, with approximately 4-5 million confirmed cases reported annually.[4] Despite being classified as a moderately malaria-endemic country, around 177 million people in Pakistan remain at risk. This is primarily due to the country's hot climate, extensive irrigation systems, fertile agricultural lands, and rainfall, which create favourable conditions for mosquitoes and facilitate the reproduction of parasites.[5] Malaria is particularly prevalent in rural areas, where poor socioeconomic conditions prevail. The two most common types of malaria in Pakistan are Plasmodium vivax and Plasmodium falciparum, with Plasmodium vivax being the dominant strain. However, the World Health Organization (WHO) has observed a six-fold increase in P. falciparum cases. When examining each province, the highest number of cases was recorded in Khyber Pakhtunkhwa (30%), followed by Sindh (26.5%), the erstwhile Federally Administered Tribal Area (FATA) (21.9%), Baluchistan (20.5%), and The Punjab with the lowest occurrence at 1.1%.[6]

Though the haematological alterations linked to malaria have been extensively studied, it is feasible

Correspondence: Dr Usama Bin Khalid, Department of Pathology, Combined Military Hospital, Dera Nawab Sahib Pakistan
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that variations in demographic settlements may also impact the documented changes.[7] According to a recent study, the white blood cell count, as well as platelets and red blood cells, decreased significantly in patients infected with malaria.[8] As well, low levels of platelets, white blood cells (WBCs), and lymphocytes were found to be significant predictors of malaria infection and could enhance malaria diagnosis and treatment. However, another study indicated elevated white blood cell levels in participants infected with malaria, in contrast to their uninfected counterparts, suggesting that the relationship between malaria and specific immune-haematological factors may be more intricate than previously believed.[9]

Many studies have been conducted to understand the relationship of malarial infection and conditions like anaemia, thrombocytopenia, and leukopenia, but no such investigation has been performed in Gilgit-Baltistan. The purpose of this study is to understand the impact of malaria on blood-related factors and identify the prevalent parasite species causing malaria in the Skardu region, located in Gilgit-Baltistan. By conducting this study, we aim to enhance our understanding of the epidemiology and seasonal changes of malaria in Skardu. Furthermore, the findings will aid in the development of effective strategies for controlling malaria and educating the local population.

METHODOLOGY

This cross-sectional study was performed at Combined Military Hospital (CMH), Skardu, Pakistan, from Jan to Dec 2022 after taking approval from Institutional Review Board (IRB), vide reference number CMH Skardu/IRB/21/02. A total of one hundred participants were recruited in the study after sample size calculation at 95% confidence interval with 5% margin of error, taking the population prevalence of 5%, which came out to be slightly less than 100.[10] Sampling was done using a non-probability consecutive sampling technique.

Inclusion Criteria: Individuals between 15 and 45 years who tested positive for malaria and exhibited various clinical symptoms were included in the study.

Exclusion Criteria: Patients below 15 and above 50 years of age, and those who were critically ill with other medical conditions, were excluded from the study.

Written consent was obtained before enrolling all patients, and their confidentiality was ensured at all levels. A trained phlebotomist drew 2 ml of venous blood from each participant in the hospital laboratory. The collected blood was then transferred into an Ethylene Diamine Tetra acetic Acid (EDTA)-lined tube. We used the blood sample to create thin and thick films for microscopic examination and automated complete blood counts (CBCs). The Sysmex XP-300 Automated Haematology Analyzer was used to estimate the blood parameters. The reference ranges for red blood cell (RBC) count, haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), platelet counts and the red blood cell distribution width (RDW) were 4.5 – 6.5×1012/L in males and of 3.8 – 5.8×1012/L in females, 13-17g/dL in male and 12-15g/dL in female, 76-96 FL, 27-32 pg,150-400×109 /L and 5-10 fL respectively.

To perform parasitological analysis, a malaria parasite antigen test using the immuno-chromatography (ICT) method was conducted, and then the results were confirmed using thin smear films created from each patient's sample. After allowing the smears to dry in air, we fixed them with methanol and applied a 10% Giemsa stain for 15 minutes. The slides were examined under a 100x oil immersion lens to identify the presence or absence of malarial parasites. For a slide to be classified as negative, no malaria parasite should be observed after scanning one hundred high-power fields (HPFs). We also identified the specific species of malaria parasites in the samples.

Statistical Package for Social Sciences (SPSS) version 23.0 was used for data analysis. Descriptive statistics, including mean and standard deviation, were determined for continuous variables. Frequency and percentage were calculated for categorical variables. Chi square and one-way ANOVA were used to find any significant difference between the groups, and $p \leq 0.05$ was taken as statistically significant.

RESULTS

Out of total patients, 64% patients were male and 36% were female. Their ages ranged from 15 to 45 years. The average age of male patients was 30.17±7.91 years, while the average age of female patients was 29.61±7.98 years. Out of the total cases, 80% were diagnosed with P. vivax malaria, 12% with P. falciparum malaria, and 8% had a mixed type of infection Figure.

Several haematological parameters were studied in the infected participants, including TLC, RBCs, platelets, Hb, haematocrit, MCV, MCH, neutrophils, and lymphocytes, as seen in Table-I.

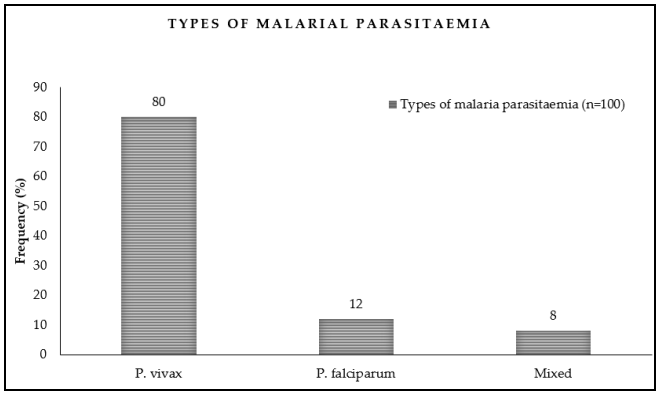

Figure: Frequency of Common Plasmodium Species at Skardu (n=100)

Table-I: Levels of Haematological Parameters in Infected Study Participants (n=100)

Haematological Parameters	(Mean±SD)
Total leukocyte count (× 109/L)	3.98±0.87
Red blood cells (× 1012/L)	4.20±0.26
Platelets (× 109/L)	124.54±29.56
Haemoglobin (g/dL)	10.93±0.85
Haematocrit (%)	39.25±3.33
Mean corpuscular volume (fL)	94.25±3.29
Mean corpuscular haemoglobin (pg.)	26.60±0.74
Neutrophils (%)	53.74±12.03
Lymphocytes (%)	24.95±6.49

The patients infected with P. vivax had less pronounced anaemia with a mean Hb 11.1±0.63 g/dl, and a significant decrease in mean platelet count of 113.36±20.94 x 109/L, respectively. Whereas Plasmodium falciparum was more strongly associated with anaemia and leukopenia. Table-II indicates a stronger connection between Plasmodium vivax and thrombocytopenia, with a *p*-value of 0.020. This information can be valuable for aiding in the diagnosis and prognosis of malaria infections.

Table-II: Basic Haematological Parameters in Study Population (n=100)

Parameter	Type of malaria			*p*-value
	P. vivax (n=80)	P. falciparum (n=12)	Mixed Malaria (n=8)	
	Mean±SD	Mean±SD	Mean±SD	
Total haemoglobin (g/dL)	11.1±0.63	9.94±0.81	9.94±1.00	0.001
Total leukocyte count (x109/L)	4.04±0.94	3.64±0.15	4.19±0.14	0.001
Platelets (x109/L)	113.36±20.94	166.92±9.88	124.54±29.56	0.020

About 58% of patients had thrombocytopenia, 36% had bicytopenia (21% with anaemia and thrombocytopenia and 15% with leukopenia and thrombocytopenia), and 6% patients had pancytopenia with p value less than 0.001 as seen in Table-III.

Table-III: Haematological Parameters in Distinct types of Malaria (n=100)

Haematological Parameters		Types of Malaria				*p* value
		P. vivax	P. falciparum	Mixed	Total	
	Thrombocytopenia	48(60.0%)	6(50.0%)	4(50.0%)	58(58.0%)	<0.001
	Anaemia + Thrombocytopenia	16(20.0%)	3(25.0%)	2(25.0%)	21(21.0%)	<0.001
	Leukopenia + Thrombocytopenia	12(15.0%)	2(16.6%)	1(12.5%)	15(15.0%)	<0.001
	Pancytopenia	4(5.0%)	1(8.3%)	1(12.5%)	6(6%)	<0.001
	Total	80 (80.0%)	12(12.0%)	8(8.0%)	100(100%)	

There was an inverse correlation between haemoglobin and platelet levels on the one hand and the likelihood of testing positive for malaria on the other, indicating that the patients who had low levels of haemoglobin or platelets were more likely to be infected with the disease. As seen in Table-IV, all patients have less pronounced anaemia with a mean Hb of 10.98 g/dl, and a significant decrease in the mean of platelets and TLC that is 120.60 x 109/L and 3.93 x 109/L, respectively.

Table-IV: Gender wise Distribution of Haematological Parameters (n=100)

Parameters	Gender		Total	*p*-Value
	Male	Female		
	Mean±SD	Mean±SD	Mean±SD	
Haemoglobin (g/dL)	10.84±0.7971	11.24±1.184	10.98±0.968	0.046
Total leukocyte count (× 109/L)	3.70±1.339	4.34±1.71	3.93±1.509	0.042
Platelets (× 109/L)	124.45±23.795	113.75±28.625	120.60±26.012	0.048

DISCUSSION

The present study demonstrates significant haematological alterations in patients with malaria, particularly anaemia, thrombocytopenia, lymphopenia, monocytosis, and eosinopenia, with more pronounced changes in Plasmodium falciparum infections. These findings highlight the diagnostic value of routine blood indices in malaria evaluation. In severe cases, it can lead to major complications and have far-reaching consequences.[11] The disease typically presents with abnormal blood test results, with anaemia and thrombocytopenia being the most common findings. The objective of the study was to examine the laboratory characteristics of individuals with malaria and analyse them in relation to existing global research.

The authors studied how malaria affects the blood characteristics of people residing in Gilgit-Baltistan. As reported by Iqbal *et al*., our study also observed similar notable variations in various red blood cell measurements among individuals infected with malaria.[12] The parasite responsible for the severe form of malaria, Plasmodium falciparum, enters and reproduces within red blood cells through a destructive cycle, which contributes significantly to the intensity and fatality of the disease, as concluded by Mkumbaye *et al*.,[13] Mace *et al*., described that the host immune system typically employs haemolytic mechanisms to eradicate infected red blood cells, potentially causing anemia.[14] The infected population in this study displayed significantly reduced levels of Hb. Surprisingly, in this study, during malaria, peripheral leukocyte (WBC) counts were found to be within the normal-to-low range, which is unexpected since one would anticipate increased WBC production during an infection. However, this study did not identify any significant changes in WBC levels among participants with malaria. One notable finding from the study was the marked decrease in platelet levels observed in individuals infected with malaria, which aligns with the findings of Kawaguchi *et al*.,[15]

In our study, we found that Plasmodium vivax, as opposed to Plasmodium, was associated with a 65% decrease in platelet counts. There are multiple theories explaining this decrease in platelets during malaria infection, as concluded in a study by Tetteh *et al*.[16] One possibility explained by Gebreweld *et al*., is the formation of blood clots in the bloodstream intermittently, leading to blockages in small blood vessels due to a decrease in number of circulating platelets.[17] Another explanation could be an immune-mediated mechanism highlighted by Mahittikorn *et al*., in which specific immunoglobulin G (IgG) produced in response to the parasite invasion binds to parasite antigens, resulting in damage to platelets. The damaged platelets are then removed from circulation.[18]

Thrombocytopenia has been identified in the literature as a reliable indicator for diagnosing malaria when accompanied by acute febrile illness. A study conducted in Ghana revealed that 87% of malaria-positive patients had Plasmodium falciparum, while 13% had Plasmodium malariae.[15,16] Our own study found that 80% of patients evaluated positive for P. vivax malaria, 12% for P. falciparum malaria, and 8% had mixed malaria with both P. vivax and P. falciparum. The variation in Plasmodium species distribution differs from one region to another, which also explains the higher occurrence of complications and fatalities associated with P. falciparum. Another systematic review and meta-analysis carried out by Kotepuri *et al*., confirmed that Plasmodium vivax was present in 57% of patients, Plasmodium falciparum in 4%, and mixed malaria in 1% of cases, whereas our study observed a higher prevalence of Plasmodium vivax.[19] In studies conducted by Bohli *et al*., also concluded that Plasmodium vivax accounted for 55% and 41%, while Plasmodium falciparum was responsible for 45% and 59% of cases, respectively.[20] In this research, it was found that 80% of malaria patients had Plasmodium vivax, while 12% had Plasmodium falciparum. The emergence of Plasmodium falciparum in Southeast Asian countries is a concerning situation that necessitates increased focus on malaria eradication efforts compared to the past.

A study in Ghana found that 48% of malaria patients had thrombocytopenia, 55% had anaemia, and 56% had leukopenia. In contrast, our study reported 58% thrombocytopenia, 21% anaemia, and 15% leukopenia among malaria-positive patients.[15] This suggests that Plasmodium vivax is more linked to thrombocytopenia, while Plasmodium falciparum is more associated with anaemia and leukopenia. Additionally, Tetteh *et al*., indicated 54% thrombocytopenia, 77% anaemia, and 9% leukopenia in malaria patients.[16] Compared to our findings, thrombocytopenia is more prevalent and anaemia less so in Gilgit-Baltistan, while leukopenia rates are similar, possibly due to increased Plasmodium malaria cases in the respective population.

The effects of malaria on blood-related factors, such as anaemia, might be explained by changes in the surface features of red blood cells (RBCs). These altered characteristics can lead to the destruction of RBCs in the spleen, resulting in reduced levels of haemoglobin, red blood cells, and haematocrit (HCT).

LIMITATIONS OF THE STUDY

In this study, we did not examine nutritional deficiencies, helminth infections, or haemoglobinopathies. Therefore, we could not determine how these factors might have influenced the observed changes in blood characteristics. Lack of information on recent medical histories, as there are many other diseases and conditions, may affect haematological values and could potentially affect the interpretation of the results.

CONCLUSIONS

Study participants diagnosed with malaria demonstrated significant alterations in multiple haematological parameters. Anaemia, thrombocytopenia, lymphopenia, monocytosis, and eosinopenia were identified as prominent indicators of infection, particularly in cases attributed to Plasmodium falciparum. Integration of these haematological abnormalities with clinical features and microscopic findings may enhance the accuracy of malaria diagnosis and inform timely therapeutic interventions.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

MA & UBK: Data acquisition, data analysis, critical review, approval of the final version to be published.

AA & QZ: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

AG: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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