Vol. 5 No. 2 (2026)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387

Print ISSN 3007-2379

Shah et al - 2026

DOI: http://doi.org/10.5281/zenodo.20504944

PREVALENCE OF MALARIAL PARASITE AND STUDY OF VARIOUS BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS BEFORE AND AFTER TREATMENT IN POPULATION OF DISTRICT BANNU

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Vol. 5 No. 2 (2026)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387

Print ISSN 3007-2379

Shah et al - 2026

DOI: http://doi.org/10.5281/zenodo.20504944

Author Details

Keywords:

Received on 03 Apr 2026

Accepted on 04 May 2026

Published on 30 May 2026

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Abstract

Malaria remains a major public health concern in Pakistan, with Plasmodium vivax predominating in most endemic areas. This study aimed to evaluate the prevalence of malaria and assess associated hematological, biochemical, and electrolyte alterations among patients in District Bannu, Khyber Pakhtunkhwa. A total of 2,500 blood samples were collected from clinically suspected cases and examined by both microscopy and rapid diagnostic tests (RDTs). All positive cases (100%) were identified as P. vivax monoinfections, with no detection of P. falciparum or mixed species. Hematological analysis revealed significant anemia (mean Hb: 9.0 g/dl), leucopenia (mean WBC: 4,100 cells/ml), and marked thrombocytopenia (mean platelets: 14,300 cells/mm^2) prior to treatment, with partial recovery post-treatment. Biochemical profiling demonstrated hypoglycemia, hyperbilirubinemia, and dyslipidemia characterized by reduced total cholesterol, HDL, and LDL levels. Liver enzymes (ALT, AST) were mildly elevated, while electrolyte analysis indicated hyponatremia, hypokalemia, and hypocalcemia before treatment, with improvement following therapy. These findings highlight that P. vivax infection in the study area is associated with substantial hematological and metabolic disturbances, emphasizing the need for timely diagnosis, appropriate management, and strengthened malaria control strategies in endemic regions.

INTRODUCTION

Malaria is a vector-borne infectious disease caused by protozoan parasites of the genus *Plasmodium* that infect peripheral blood (1). It is not only one of the most prevalent tropical diseases worldwide but also poses a major threat to public health and the economic development of many countries (2).

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Despite advancements in medical science, malaria remains a significant global health challenge, causing an estimated one million deaths annually (3). Over one billion people live in regions with a high risk of malaria transmission (4). In 2010, an estimated 3.3 billion people were at risk of malaria. Among all geographical regions, sub-Saharan Africa bears the highest burden, accounting for approximately 81% of malaria cases and 91% of related deaths in that year. Children under five years of age and pregnant women are the most severely affected (5). Malaria is more prevalent in rural areas, where access to healthcare is limited. As a result, many cases go undiagnosed and unreported, suggesting that the actual number of malaria patients may be significantly higher than documented (6).

Malaria is endemic in various regions of the world, including parts of the Americas, most of Asia, large areas of Africa, and especially regions located near the equator (7). In Pakistan, both *Plasmodium vivax* and *Plasmodium falciparum* are prevalent (8). According to the World Health Organization (9), approximately 97% of the Pakistani population around 150 million people is at risk of malaria, with an estimated annual burden of 1.6 million cases.

Malaria transmission in Pakistan is highly seasonal and prone to epidemic outbreaks in specific regions, particularly Khyber Pakhtunkhwa, Balochistan, and Sindh provinces. The peak transmission season typically occurs from September to November, following the monsoon rains. A secondary, shorter transmission period occurs in spring (March–April), though most of these cases are believed to be relapses or delayed presentations of *P. vivax* infections acquired during the monsoon season. *P. vivax* accounts for roughly 75% of malaria cases, while *P. falciparum* contributes around 25% (10). As a tropical and largely agricultural country with a majority of its population living in rural areas and below the poverty line, Pakistan is particularly vulnerable to malaria. Several factors contribute to the increasing incidence of malaria, including poverty, environmental degradation, and the widespread emergence of chloroquine-resistant *P. falciparum*. Additionally, warmer autumns that prolong the transmission period, reduced vector control efforts, extensive

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irrigation systems, monsoon rainfall, and large-scale agricultural activities create ideal breeding conditions for malaria vectors (11).

Pakistan is considered hyperendemic for malaria; however, accurate and comprehensive data on its prevalence remain limited (12). While *Plasmodium vivax* remains the dominant species causing malaria in the country, the incidence of *P. falciparum* has been steadily increasing and now accounts for approximately 35–40% of malaria cases (13). Given this concerning trend, the present study was undertaken to evaluate the current malaria situation in the local population of Bannu District.

1. Materials and Methods

2.1. Description of the Study Area

The study was conducted in Bannu District (32°43'–33°06' N; 70°22'–70°57' E), located in Khyber Pakhtunkhwa (KPK), Pakistan (Fig. 1). The Kurram and Gambila rivers flow through the district, providing water for irrigation and unintentionally creating ideal breeding sites for malaria vectors. The mean daily temperature in the area ranges from 10.8°C to 32.9°C. Bannu experiences two distinct rainy seasons: one in March (spring) and the other during the summer monsoon period in July and August (Ministry of Health, Pakistan, 2010).

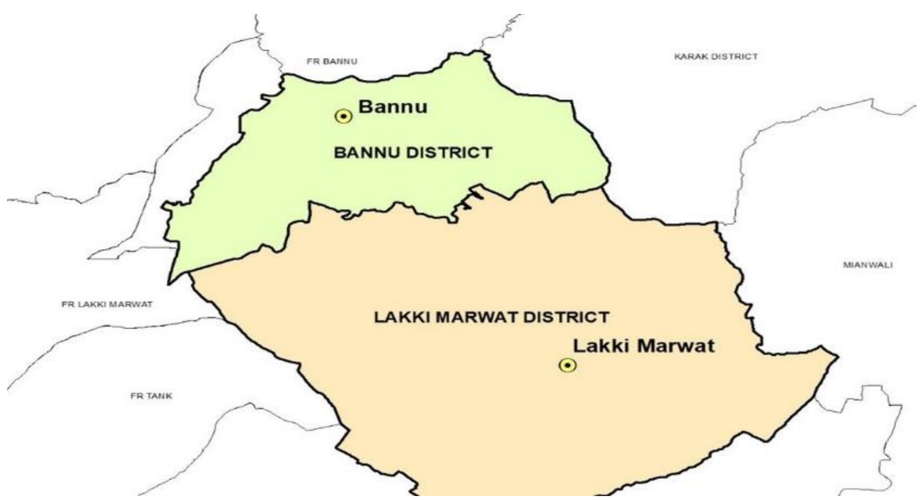

Fig. 1. Map of Pakistan showing the study area in Khyber Pakhtunkhwa

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2.2. Sample Collection

A total of 2500 blood samples were collected from suspected malaria patients visiting DHQ hospital Bannu and other local healthcare facilities across various areas of District Bannu. The study population included 1920 males and 580 females, reflecting a higher incidence of malaria in the male population. Blood samples were obtained through venipuncture using sterile techniques and were immediately transferred to EDTA and plain tubes for hematological and biochemical analyses, respectively. In addition, blood films were prepared on clean glass slides, air-dried, and stained using standard Giemsa staining protocol for microscopic identification of malarial parasites. All sample collections were carried out following standard biosafety protocols, and informed verbal consent was obtained from the patients prior to collection.

2.3. Microscopic Examination and Hematological Analysis

For microscopic diagnosis of malaria, both thick and thin blood films were prepared from each patient sample. The thin smears were fixed with absolute methanol for 30 seconds after air-drying. All smears were stained using freshly prepared 10% Giemsa stain, prepared by mixing 1 ml of stock solution with 9 ml of phosphate buffer (pH 7.2). After staining, the slides were gently rinsed with tap water and allowed to dry in an upright position. The prepared slides were then examined under a light microscope using a 100× oil immersion objective lenses. For quality assurance, each new batch of Giemsa stain was validated using a positive control smear. Parasite density was assessed by counting the number of parasites per thick film field, and all smears were double-checked independently. Smears were considered negative only when no parasites were detected across 100 high-power fields.

In addition to microscopy, hematological parameters were evaluated using the ERBA XL-200 Hematology Analyzer. EDTA-treated blood samples were gently mixed on a rotator for 10–15 minutes to ensure homogeneity. The samples were then aspirated into the analyzer via a sample probe, and results were displayed within 20 seconds. A complete blood count (CBC) was performed

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using fresh blood to measure critical indicators such as hemoglobin concentration, total leukocyte count (TLC), and platelet count. Normal reference ranges were as follows: hemoglobin - 13 to 18 g/dL for males and 11 to 16 g/dL for females; TLC – 4,000 to 11,000 cells/cmm; and platelet count – 150,000 to 450,000 cells/cmm.

2.4. Biochemical and Electrolyte Analysis

Biochemical and electrolyte parameters of the blood samples were assessed using standard clinical laboratory procedures and automated analyzers. Blood was collected in clot-activator tubes, and serum was separated by centrifugation. The serum was then subjected to biochemical analysis using an Automated Chemistry Analyzer (Microlab 300 LX), which measured levels of liver enzymes (ALT and AST), bilirubin, cholesterol, HDL, LDL, and glucose. Reagents and calibrators were used according to the manufacturer's instructions, and absorbance readings were obtained spectrophotometrically. For liver function assessment, ALT and AST were analyzed using reagent mixtures with appropriate serum volumes, and results were interpreted using reference ranges of up to 42 U/L for ALT and 40 U/L for AST. Total bilirubin was determined through sequential reagent addition followed by incubation, with a normal reference range of 0.2–1.0 mg/dL. Cholesterol levels were estimated using enzymatic methods, with HDL and LDL measured directly through incubation of serum with respective reagents at 37°C, and values interpreted using standard clinical ranges. Random blood glucose (RBS) levels were analyzed by mixing serum with prepared reagent, incubating the mixture, and measuring absorbance after 10 minutes. Normal RBS and fasting blood glucose (FBS) ranges were considered as 80–140 mg/dL and 70–110 mg/dL, respectively. Electrolyte levels, including sodium, potassium, calcium, phosphate, and bicarbonate, were measured using a fully automated arterial blood gas (ABG) and electrolyte analyzer. The system required 200 μL of serum per test and provided results based on internal calibration. The following normal reference values were used for interpretation: sodium (135–145 mmol/L), potassium (3.6–5.0 mmol/L), calcium (8.6–10.2 mg/dL), phosphate (2.7–4.5 mg/dL in adults; 4.5–6.7 mg/dL in children), and

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bicarbonate (22–28 mEq/L for arterial, 24–30 mEq/L for venous samples). All analyses were conducted in duplicate to ensure precision and reliability of results.

2. RESULTS

3.1. Prevalence of Malaria Parasite

Microscopic examination of 2,500 blood samples collected from malaria-infected individuals in District Bannu revealed that **100% of cases were positive for** *Plasmodium vivax*. No cases of *P. falciparum* or mixed infections were detected in the study sample, confirming *P. vivax* as the dominant malarial species in the area.

3.2. Diagnostic Tools Performance

Both Rapid Diagnostic Tests (RDTs) and microscopic examination were used for parasite detection. All RDT-positive cases were confirmed by microscopy. However, in cases with low parasitemia, microscopy occasionally yielded false-negative results, indicating its reduced sensitivity at low parasite densities compared to RDTs.

3.3. Blood Glucose Levels

The mean blood glucose level in healthy individuals was **140 mg/dl,** whereas malaria patients had a significantly lower average of **68 mg/dl** before treatment. After treatment, the average blood glucose increased to **120 mg/dl,** approaching but still slightly below the normal range. This consistent hypoglycemia was observed across all age groups, even before the initiation of therapy.

3.4. Bilirubin Levels

Total serum bilirubin was markedly elevated in malaria patients, with an average of **5.0 mg/dl** before treatment compared to the normal average of **1.0 mg/dl**. After treatment, bilirubin levels decreased

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substantially to **0.7 mg/dl**, indicating recovery of liver function. The elevation was present even in patients infected solely with *P. vivax*.

3.5. Changes in Plasma Lipids and Lipoproteins

Total cholesterol levels were significantly reduced before treatment **(85 mg/dl)** compared to the normal average **(200 mg/dl)**, consistent with hypocholesterolemia. Following treatment, cholesterol levels improved to **166 mg/dl** but did not fully reach the normal range. HDL cholesterol decreased slightly from the normal **50 mg/dl** to **44 mg/dl** before treatment and improved to **49 mg/dl** after treatment. LDL cholesterol showed a more pronounced decrease from the normal **150 mg/dl** to **40 mg/dl** before treatment, rising to **140 mg/dl** post-treatment.

3.6. Liver Enzymes (ALT and AST)

Before treatment, AST levels averaged **53 u/L**, slightly higher than the normal **40 u/L**, and decreased to **46 u/L** post-treatment. ALT levels were similarly elevated from a normal **45 IU/L** to **55 IU/L** before treatment, later declining to **49 IU/L** after therapy. These mild increases suggest that malaria had a limited effect on liver enzyme levels in most patients.

3.7. Electrolyte Changes

Sodium levels dropped from the normal average of **145 mmol/L** to **78 mmol/L** before treatment, improving to **124 mmol/L** after treatment, indicating a trend toward correction of hyponatremia. Potassium levels decreased from the normal **5.0 mmol/L** to **2.3 mmol/L** before treatment, later increasing to **4.2 mmol/L**. Calcium levels were reduced from a normal **10 mg/dl** to **6.2 mg/dl** pre-treatment, rising to **9.0 mg/dl** post-treatment. Plasma inorganic phosphate (PO_4) decreased from the normal **7.01 mg/L** to **5.03 mg/L** before treatment and further dropped to **4.04 mg/L** after treatment. Bicarbonate (HCO_3^-) levels fell from the normal **75 mEq/L** to **30 mEq/L** before

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treatment and to **22 mEq/L** after treatment, although the changes were not statistically significant in clinical terms.

3.8. Hematological Parameters

Hemoglobin levels averaged **9.0 g/dl** before treatment compared to the normal **16 g/dl**, later increasing to **11 g/dl** after treatment, indicating anemia in most patients. White blood cell counts dropped from a normal **11,000 cells/ml** to **4,100 cells/ml** before treatment, improving to **5,700 cells/ml** post-treatment, reflecting leucopenia in the majority of cases. Platelet counts showed severe thrombocytopenia, with pre-treatment averages of **14,300 cells/mm²** compared to the normal **450,000 cells/mm²**. After treatment, platelet counts improved to **193,000 cells/mm²**, though still below normal in many patients.

Table.1. Blood parameters in Plasmodium Vivax before and after treatment.

Parameters	Patients	Normal Average level	Average lever before treatment	Average level after treatment
Blood glucose level (mg/dl)	2500	140	68	120
Bilirubin level (mg/dl)	2500	1	5	0.7
Cholesterol level (mg/dl)	2500	200	85	166
AST level (u/L)	2500	40	53	46
ALT level (IU/L)	2500	45	55	49
HDL level (mg/dl)	2500	50	44	49
LDL level (mg/dl)	2500	150	40	140
Na level (mmol/L)	2500	145	78	124
K level (mmol/L)	2500	5	2.3	4.2

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Ca level(mg/dl)	2500	10	6.2	9
PO4 level (mg/L)	2500	7.01	5.03	4.04
HCO3 Level (mEq/L)	2500	75	30	22
Hb level (g/dl)	2500	16	9	11
WBCs level (cells/ml)	2500	11000	4100	5700
Platelets level (cells/mm^2)	2500	450000	14300	193000

4. Discussion

This study investigated the prevalence of malaria parasites and their effects on various biochemical and hematological parameters in the population of District Bannu, with measurements taken before and after treatment. The findings demonstrate that *Plasmodium vivax* was the sole and predominant malarial species in the area, affecting multiple physiological systems and producing measurable biochemical and hematological alterations. The observed changes highlight both the direct pathogenic effects of the parasite and the partial reversibility of these effects following antimalarial treatment. Microscopic examination of 2,500 samples confirmed *P. vivax* as the only species present, accounting for 100% of infections. This finding is in agreement with previous reports from other regions of Pakistan, where *P. vivax* is more prevalent than *P. falciparum*, especially in the northwestern belt (14). The absence of *P. falciparum* or mixed infections in this study may be related to ecological, climatic, or vector distribution factors specific to Bannu. Both microscopy and rapid diagnostic tests (RDTs) performed well in diagnosis; however, low-parasitemia cases occasionally produced false-negative microscopy results, confirming earlier observations that RDTs may have superior sensitivity in low-density infections (14).

A marked hypoglycemia was detected in malaria patients prior to treatment, with average blood glucose dropping from the normal 140 mg/dl to 68 mg/dl. This decrease can be attributed to increased glucose consumption by both host immune responses and the parasite itself, as *Plasmodium* relies heavily on host glucose for its energy metabolism (15). Even after treatment, glucose levels rose

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only to 120 mg/dl, remaining slightly below normal, suggesting that recovery of metabolic balance may lag behind parasite clearance. Persistent mild hypoglycemia post-treatment could also be influenced by nutritional deficiencies or ongoing systemic inflammation. Bilirubin levels showed a dramatic rise from 1.0 mg/dl in healthy individuals to 5.0 mg/dl in infected patients, before decreasing to 0.7 mg/dl after therapy. This elevation likely results from increased hemolysis of parasitized red blood cells and impaired hepatic clearance of bilirubin during acute infection (16). The normalization of bilirubin after treatment indicates that liver function impairment in *P. vivax* malaria is generally reversible, though the transient hyperbilirubinemia may contribute to the clinical jaundice observed in some patients.

The study also confirmed the occurrence of malaria-associated hypocholesterolemia, with total cholesterol falling from 200 mg/dl to 85 mg/dl pre-treatment. LDL cholesterol showed a pronounced drop from 150 mg/dl to 40 mg/dl, while HDL declined modestly. After treatment, partial recovery was observed, but lipid levels did not fully return to normal. These findings are consistent with previous reports (17) attributing the changes to increased lipid utilization by the parasite for membrane synthesis during schizogony, as well as cytokine-mediated alterations in lipid metabolism. AST and ALT levels showed only mild elevation before treatment, with averages of 53 u/L and 55 IU/L, respectively, compared to normal values of 40 u/L and 45 IU/L. These changes were largely reversible, suggesting minimal hepatocellular injury in most *P. vivax* cases. Previous studies have also reported that liver enzyme elevations are more prominent in *P. falciparum* infections or in severe *P. vivax* malaria complicated by hepatic dysfunction (18).

Significant electrolyte imbalances were noted, including hyponatremia (78 mmol/L), hypokalemia (2.3 mmol/L), and hypocalcemia (6.2 mg/dl) before treatment. These abnormalities improved post-treatment but did not always return to normal ranges. Such changes may be due to increased renal losses, shifts between intracellular and extracellular compartments, and parasite-induced alterations in cell membrane transport. Interestingly, bicarbonate levels decreased both before and after treatment, though not to a clinically critical degree, suggesting possible persistent metabolic acid-

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Online ISSN 3007-2387 Print ISSN 3007-2379

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DOI: http://doi.org/10.5281/zenodo.20504944

base imbalance. Hematological alterations were among the most profound findings. Anemia was highly prevalent, with hemoglobin levels dropping from 16 g/dl in healthy controls to 9 g/dl in infected patients, improving to 11 g/dl after treatment. The anemia likely results from a combination of hemolysis, splenic sequestration, and bone marrow suppression. Severe thrombocytopenia (14,300 cells/mm^2) was common, with partial recovery post-treatment (193,000 cells/mm^2). Leucopenia was also frequent, possibly reflecting bone marrow suppression or redistribution of leukocytes to the spleen and other tissues. These findings are consistent with previous studies identifying anemia and thrombocytopenia as hallmark hematological features of *P. vivax* malaria (19). The results indicate that *P. vivax* malaria, often considered less severe than *P. falciparum*, can produce significant biochemical and hematological abnormalities. Many parameters showed only partial normalization after treatment, highlighting the need for extended follow-up and supportive care beyond antimalarial therapy. Correcting nutritional deficiencies, monitoring liver function, and managing electrolyte imbalances should be integral to post-treatment recovery plans. A limitation of this study is that it focused solely on *P. vivax* cases in one district, which may not reflect parasite distribution in other regions of Pakistan. Longitudinal studies with larger geographic coverage could better assess seasonal and regional variations. Additionally, including follow-up measurements beyond the immediate post-treatment phase would clarify the timeline for complete biochemical and hematological recovery.

3. Conclusion

This study confirms *Plasmodium vivax* as the sole malarial species prevalent in District Bannu. Malaria significantly impacted multiple biochemical and hematological parameters, including marked hypoglycemia, hyperbilirubinemia, hypocholesterolemia, electrolyte imbalances, anemia, leucopenia, and severe thrombocytopenia. Post-treatment, most parameters showed substantial improvement, though several remained below normal ranges, indicating partial recovery and the need for continued monitoring. These findings highlight the systemic effects of *P. vivax* infection and

Online ISSN 3007-2387 Print ISSN 3007-2379

DOI: http://doi.org/10.5281/zenodo.20504944

emphasize the importance of timely diagnosis, effective treatment, and post-therapy follow-up to ensure full physiological restoration.

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https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387

Print ISSN 3007-2379

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