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Impact of mixed versus monospecies malaria on organ dysfunction and hospital course: A retrospective cohort study from Karachi, Pakistan

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Impact of mixed versus monospecies malaria on organ dysfunction and hospital course: A retrospective cohort study from Karachi, Pakistan

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Abstract

Background: Mixed malaria refers to simultaneous infection with two or more Plasmodium species, most often P. vivax and P. falciparum. While monospecies infections are well studied, the comparative complication profiles of mixed versus monospecies malaria remain poorly characterized in the literature search of South East Asia. Prior reports suggest heightened severity, in mixed malaria infection, contributing to triage bias and resource strain. This study aims to assess the impact of mixed malaria on organ dysfunction and hospital course compared to mono-species malaria in a tertiary care hospital.

Methods: A retrospective cohort study was conducted at Liaquat National Hospital, Karachi (Jan 2023–Jan 2024). Malaria was confirmed by immunochromatographic assays and peripheral blood film microscopy. Adults aged 18–70 years with mixed or monospecies infections were included; chronic comorbidities were excluded except diabetes, hypertension, and ischemic heart disease.

Patients were categorized into P. vivax, P. falciparum, and mixed cohorts. Organ dysfunctions or complications were defined as hematological, renal, hepatic, pulmonary, neurological, or cardiac complications, hospital course included, length of hospital stay and mortality. Data were analyzed using SPSS v27.

Results: Among **159 patients** (mean age 42.5 ± 17.6 years)**, P. vivax malaria** was most frequent (55.3%), followed by **Mixed malaria** (28.9%) and **P**. **falciparum malaria** (15.7%). All patients developed **complications**, averaging **4.3 ± 1.4**, with 68.6% experiencing ≥4. Common findings included **thrombocytopenia** (86.2%), **anemia** (59.1%), **hyponatremia** (47.8%), **hepatic injury** (45.3**%**)**, metabolic acidosis** (43.4%), and **acute kidney injury** (16.4%). Odds of **thrombocytopenia** were higher in **P. Vivax** versus **Mixed malaria** (odds ratio [OR] 3.3, 95% confidence interval [CI] 1.1–10.0)**. P falciparum** carried greater risk of **acute kidney injury** (OR 3.2, 95% CI 1.1–9.5), **hepatic injury** (OR 3.7, 95% CI 1.3–10.5), and **metabolic acidosis** (OR 2.7, 95% CI 1.1–7.5) compared to **Mixed infections**.

Conclusion: Mixed malaria infections were associated with reduced severity compared to mono-species infections. P. falciparum carried the greatest risk of renal, hepatic, and metabolic complications, while P. vivax was strongly linked to thrombocytopenia. These findings suggest mixed infections may exert a modulatory effect on disease severity, with implications for triage and management in endemic regions.

Key Words: Mono species malaria., Mixed malaria ,Organ dysfunction /complications

Background

Malaria remains a major public health challenge worldwide, particularly in tropical and subtropical regions. In Pakistan, the disease is endemic with seasonal peaks between September–December and April–May, closely linked to agricultural cycles (1). Longitudinal surveillance from the province of Sindh where Karachi is situated, confirms post-monsoon peaks and flood-related amplification (2). Urban data from Karachi also show strong correlations between rainfall, humidity, and malaria incidence (3). According to the World Health Organization (WHO) Disease Outbreak News, suspected malaria cases in Pakistan rose from 2.6 million in 2021 to 3.4 million in 2022, reflecting an excess of approximately 0.8 million cases. following the 2022 floods (1).

Mixed malaria infections—defined as the simultaneous presence of two or more Plasmodium species in a single host—are of particular concern worldwide (5) A systematic review and meta-analysis estimated the prevalence of mixed malaria infections in Pakistan at 3.98%, with P. falciparum and P. vivax co-infection being the most frequent pairing. This finding highlights the fact that, although mixed infections represent a minority of cases, they remain epidemiologically significant in endemic regions, particularly given the dominance of *falciparum–vivax* co-transmission (4). Despite this significance, mixed infections remain underdiagnosed. Conventional light microscopy frequently fails to detect low-density secondary species, leading to misclassification as mono species malaria (5). Rapid diagnostic tests (RDTs), however, improve detection rates and may provide a more reliable point-of-care solution, (6), whereas polymerase chain reaction (PCR) remains the gold standard for detecting mixed infections, especially at low parasite densities and in epidemiological studies (7).

Clinically, Mixed malaria presents a complex picture. Systematic reviews and meta-analyses indicate that it can present as a severe illness, with complications such as anemia, pulmonary failure, renal impairment, hepatitis, cerebral malaria, convulsions, disseminated intravascular coagulation, and multi-organ failure affecting ~9% of patients overall (8). Several mechanisms may contribute to, this enhanced severity: co-infection increases parasite biomass, exacerbating anemia and metabolic stress;(5) simultaneous activation of divergent immune pathways produces dysregulated responses with overlapping pro-inflammatory cytokine surges;(41) and diagnostic delays or misclassification often enrich hospital datasets with patients presenting later and with more complicated disease (4). Antimalarial drug resistance further alters treatment efficacy and severity, particularly when resistant P. falciparum strains are involved (9). Host genetic diversity, including sickle cell trait, glucose-6-phosphate dehydrogenase (G6PD) deficiency, and human leukocyte antigen (HLA) polymorphisms, modulates susceptibility and disease course (10), while parasite genetic variation contributes to differences in virulence and clinical manifestations (11). Prior malaria exposure and acquired immunity also critically shape severity outcomes (12). Pregnancy and childhood are a well-recognized risk factor for heightened complications (13,14). Vector diversity contributes to the ecological basis for mixed infections (15), while relapse biology links P. falciparum gametocyte carriage to subsequent P. vivax episodes, adding complexity to clinical management (16). On the other end of spectrum, there is some literature evidence suggesting that, Mixed malaria infections may attenuate disease severity by suppressing the pathogenicity of one species, as shown in experimental models (17) and supported by human data reporting variable but occasionally reduced severity (18). Elevated interleukin-10 (IL-10) in *P. vivax* and mixed infections suppresses tumor necrosis factor alpha (TNF-α) and interferon gamma

(IFN-γ), limiting immunopathology such as anemia and renal injury (19), while frequent relapses provide repeated low-level antigenic exposure that conditions the immune system toward less inflammatory responses (20). Activation of the adenosine signaling pathway in P. Vivax downregulates nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and reduces pro-inflammatory cytokine release, promoting immune tolerance (21). Expansion of Regulatory T Cells (CD4+CD25+FoxP3+ Tregs) further suppresses effector activity and modulates immunity (19). Plasmodium. Vivax also stimulates T cell subsets like Gamma Delta (γδ T) cells (22) repeated malaria exposure in endemic regions shifts them toward regulatory/tolerant functions, which helps attenuate severity in mixed infections. (23). In contrast, P. falciparum infection is marked by hyperinflammation, with excessive Cytokines like TNF-α, interleukin-6 (IL-6), and IFN-γ driving endothelial activation, capillary leak, and organ damage (24,25). Cross-reactive immunity in mixed infections reduces parasitemia, limits immune exhaustion, and sustains protective responses (26).

The need for this study arose from the fact, that while monospecies infections have been extensively studied, the clinical impact of mixed infections is less well characterized, particularly in Pakistan and South Eastern countries. Existing literature from endemic regions has largely focused on prevalence and descriptive epidemiology, with limited attention to comparative complication profiles. This study addresses that gap. By applying WHO-defined criteria for severe malaria and conducting multivariate analyses, we provide distinct insights into the attenuating effect of mixed infections on clinical severity. These findings are relevant not only to Pakistan but also to other endemic regions where Mixed malaria infections are under-recognized, and they contribute to the broader international discourse on malaria pathophysiology and management.

Methods

Study design, settings and duration

This retrospective cohort study was conducted in the Department of Internal Medicine at Liaquat National Hospital and Medical College, Karachi, Pakistan. Data was reviewed and retrieved for period of January 2023 to January 2024. While we reviewed, extracted and completed the manuscript preparation during February 2025 to May, 2026.

Inclusion & exclusion criteria

Participants were aged 18–70 years with confirmed malaria, diagnosed using the immunochromatographic test (ICT) Malaria kit (Accu Check P. falciparum/PAN Rapid Test). Peripheral blood film microscopy confirmed the pairing type (P. vivax + P. falciparum). Exclusion criteria were chronic kidney or liver disease, malignancies, hematologic disorders, autoimmune conditions however ischemic heart disease diabetes mellitus and Hypertension were not excluded, as these comorbidities are highly prevalent in malaria-endemic populations and contribute to the real-world clinical spectrum of disease, thereby reflecting the true burden encountered in clinical practice.

Sample size

Previous study showed that 42.06% malaria cases developed multi-organ dysfunction (56). At 95% confidence interval and margin of error of 8%, a sample size came out to be 145. Sample size calculation was performed using online available calculator Open-Epi.

patients with complete data were included.

Data extraction procedure, study groups and outcome variable.

Data were extracted from hospital records, using a standardized tool, anonymized, and cross-verified. Patients were categorized into three cohorts: P. vivax mono-infection, P. falciparum mono-infection, and Mixed-species malaria. The primary outcome was malaria-related complications also labeled as organ dysfunction. The extracted data included age, gender, presenting features, occupational settings, presenting features, vital signs, in-hospital complications, hospital course and final outcomes (alive/dead).

Operational definitions

Organ dysfunction or complication was operationally defined as**,** hematological, renal, hepatic, pulmonary, neurological, and cardiac complications. WHO definitions were applied to define these complications: Severe anemia — hemoglobin ≤8 mg/dL; Hemolytic anemia — hemoglobin fall with lactate dehydrogenase (LDH) >300 U/L and/or indirect bilirubin >1.5 mg/dL; Thrombocytopenia — platelets <150×10⁹/L; Acute kidney injury (AKI) — creatinine rise ≥0.3 mg/dL within 48 hours or ≥1.5× baseline within 7 days ; Hepatic dysfunction — bilirubin >3 mg/dL with alanine aminotransferase/aspartate aminotransferase (ALT/AST) >3× upper limit; Pulmonary complications — respiratory distress with bilateral infiltrates, oxygen saturation <93% on room air; Cerebral malaria — coma >1 hour post-seizure or hypoglycemia correction, with parasitemia and no alternative cause.

Hospital course, included the length of stay, recovery or morbidity

Statistical analysis

Data were analyzed using SPSS v27. Descriptive statistics summarized demographics and clinical features. Categorical variables were compared using chi-square, continuous variables with

independent t-tests. Multivariate logistic regression identified predictors of complications, adjusting for confounders. Significance was set at $p<0.05$.

Results

Vital sign parameters recorded at presentation

Table 1 depicts vital signs at presentation. The mean systolic blood pressure was 114.7±12.9 mmHg, and the mean diastolic pressure was 73.8±10.6 mmHg. Heart rate averaged 84.1±16.2 beats per minute, while respiratory rate was 18.5±1.8 breaths per minute. Peripheral oxygen saturation (SpO_2) measured 97.7±1.4% on average, and body temperature averaged 97.8±0.7 °F. Temperature, blood pressure, and oxygen saturation showed no significant difference among malaria type.

INSERT HERE TABEL 1

Summary of baseline features

Table 2. Summarizes baseline features of 159 malaria patients. The mean age was 42.5 ± 17.6 years. Most were diagnosed with P. vivax (55.3%), followed by mixed malaria (28.9%) and P. falciparum *(*15.7%). Age distribution showed peaks in the 20–29 and 60–70-year groups, each accounting for 21% of cases. Males comprised two-thirds of the cohort. Over half were employed in indoor occupations (office workers, teachers, homemakers), while the remainder worked outdoors. Fever was reported in 95% of patients, with vomiting and body ache next most common. Respiratory symptoms (cough, shortness of breath) and gastrointestinal/pain complaints (epigastric pain, headache) occurred in fewer than one-third. The mixed malaria group had a higher

mean age (52 years; 25 patients, 52.2%), whereas younger patients predominated in mono-infections: P. vivax 47 (53.4%) and P. falciparum 17 (68%) compared to mixed.

INSERT HERE TABLE 2

All of the patients experienced at least one complication. The average number of complications was 4.3±1.4. Majority had four or more complications (68.6%). None of the patient suffered from shock or respiratory failure during the hospital stay. The most common finding among the participants was thrombocytopenia, present in 86.2% of individuals, followed by anemia at 59.1%. Hyponatremia and metabolic acidosis were observed in 47.8% and 43.4% of the population, respectively. Hepatic injury was reported in 45.3% of participants. Hemolytic anemia was seen in 23.3% and leukopenia was present in 23.9% of individuals. Renal complications were present in 16.4% patients. Coma was developed in only one patient who had mixed malaria.

Comparison of complications among different Malaria cohorts

Figure 1. displays a comparison of complications among different Malaria cohorts. Frequency of anemia (p=0.255), leukopenia (p=0.287) was not significantly different among three types of malaria diagnosis. Frequency of thrombocytopenia (p=0.011), hepatic injury (p=0.014), renal complication (p=0.001), hyponatremia (p=0.003) and metabolic acidosis (p=0.05) was significantly different among three types of malaria diagnosis.

INSERT HERE FIGURE 1

Univariate associations between complications and malaria diagnosis

Univariate associations between complications and malaria diagnosis are presented in Table 3.

Anemia: Patients who developed anemia had lesser chances of having Vivax malaria as opposed to mixed malaria (OR 0.6; 95% CI: 0.3-1.2) even though this could not be concluded statistically significantly. Also, there wasn't any significant difference in the rates of P.falciparum against Mixed malaria (OR 0.8; 95% CI: 0.2-3.8). Sociodemographic factors were significant: older age (>40 years) increased odds of anemia (OR 3.0, 95% CI: 1.1–1.4), while females were disproportionately affected (OR 0.1, 95% CI: 0.04–0.2).

Leukopenia: Leukopenia, was not found to have a significant association with P. vivax malaria (OR 1.3; 95% CI 0.6-2.9) or P. falciparum malaria (OR 0.5; 95% CI: 0.1-4.1) (Table 3).

Thrombocytopenia: Thrombocytopenia was present, 3.3 times more in of P. vivax malaria comparative to Mixed malaria (OR 3.3; 95% CI: 1.1-10). However, no significant association was seen in P.falciparum malaria against Mixed malaria (OR 0.6; 95% CI: 0.2-1.9).

Renal complication: There is a 3.2-fold higher rate of renal complications in P.falciparum malaria against Mixed malaria (OR 3.2; 95% CI: 1.1-9.5) while P.vivax malaria does not have any significant relation (OR 0.5; 95% CI: 0.2-1.4).

Liver disease: Liver disease was strongly correlated with an increased possibility of P. falciparum malaria compared to Mixed malaria (OR=3.7, 95% CI: 1.3-10.5). A weak association was observed with the cases of Vivax malaria (OR=0.9, 95% CI: 0.4-1.9).

Hyponatremia: Hyponatremia was linked to fewer chances of P. vivax malaria compared to Mixed malaria (OR=0.4, 95% CI: 0.2-0.9), which means that there is a significant drop of approximately by 60% of chance. However, low sodium level showed no correlation with P.falciparum malaria (OR=2.09, 95% CI: 0.6-6.1)

Metabolic acidosis: Metabolic acidosis was found to be correlated with 2.7 times higher probability with Falciparum malaria compared to Mixed malaria (OR=2.7, 95% CI: 1.1-7.5). A weak association was found with Vivax malaria (OR=1.0, 95% CI: 0.5-2.1).

Hospital course and outcomes

Average hospital stay was 3.1 ± 2.5 days (range 1–20). Median stay was 2 days (IQR 2–3) for P.falciparum, 3 days (IQR 1.5–5.5) for P-vivax, and 3 days (IQR 2–4) for Mixed malaria cases, with no significant difference (p=0.053).All patient were discharged home fully recovered except for one mortality in a 29-year-old with P.vivax malaria, non-ST elevation myocardial infarction and a low Glasgow Coma Scale (GCS) score of 3/15 at presentation.

INSERT HERE TABLE 3

Association of Comorbid Conditions with complications.

Table 4. depicts the association of complications with age, gender, hypertension, diabetes, ischemic heart disease. Among all predictors, anemia was found to be linked to age significantly, with a higher risk among patients of more than 40 years with an odds ratio (*2.1(1.1-4.1). Anemia was also associated with gender, with lower odds among males, (*0.1(0.04-0.2) than females. Odds of renal complications were significantly higher in patients with hypertension (**6.1 (2.1–17.3) diabetes, (**7.1 (2.5–20.2), and ischemic heart disease (**6.4 (1.6–25.5). The likelihood of hyponatremia was significantly higher in patients aged >40 years, (*1.9(1.0 - 3.6). Metabolic acidosis was found to be associated with age with the odds ratio of, (*2.39 (1.25 - 4.57) gender, * 0.5 (0.2 - 0.9) showing males at a lower risk of metabolic acidosis than females.

Hypertension and diabetes were also significantly associated with metabolic acidosis showing odds ratios of (*2.3 (1.2-4.7) and diabetes (*3.0 (1.3-6.9).

INSERT HERE TABLE 4

Discussion

Mixed malaria arises in regions with diverse vectors enabling co-transmission through simultaneous or sequential exposures (15), or when dormant P. vivax hypnozoites relapse during or after new P. falciparum infection (16,27). In this study cohorts, P. vivax mono-infection was most frequent (55.3%), followed by Mixed infections (28.9%) and P. falciparum mono-infection (15.7%). These proportions differ from national meta-analysis estimates reporting higher P.falciparum prevalence (16.29%) and lower Mixed infection prevalence (3.98%) (4), This gap can be likely reflecting study design, diagnostic methods, and timing: our hospital-based sample was collected post-flood (2023–2024) using malarial parasite (MP) ICT kits plus peripheral blood film analysis, whereas national surveys relied on microscopy alone (4). Diagnosis of mixed malaria itself influences admission, as physicians are more likely to hospitalize such patients, enriching tertiary hospital datasets with severe cases (4,5). Hospital studies further demonstrate that double and triple mixed infections are detected more frequently in tertiary centers than in community surveys, underscoring referral bias (18,28). Diagnostic misclassification additionally enriches hospital datasets with severe mixed cases (5). Thus, the high complication burden observed in our malaria cohorts, where every patient had at least one complication, is best explained by referral and admission bias, consistent with hospital-based studies showing that Mixed infections are disproportionately detected in tertiary centers and associated with severe outcomes (18,28).

Thrombocytopenia: Thrombocytopenia was strikingly observed in 93% of P. vivax, 80.4% of Mixed, and 75% of P. falciparum cases. Odds ratios showed a significant association for P.vivax versus Mixed, but not for P.falciparum versus Mixed malaria. This aligns with meta-analyses confirming P. vivax as more strongly linked to severe thrombocytopenia than P. falciparum (29). Ultrastructural studies reinforce this, demonstrating direct platelet–parasite interactions, with P. Vivax parasites visualized inside platelets and pseudopodia attaching to parasitized erythrocytes, supporting platelet-mediated cytolysis (30). unlike P.vivax, falciparum does not directly invade platelets; rather, its interactions are mediated indirectly via platelet factors, diluting platelet–parasite contact in mixed infections (30,31). Species competition may also lower parasite densities, reducing opportunities for platelet invasion and clearance (32)**.**

Anemia was frequent across species: 64.0% in P. falciparum, 67.4% in Mixed, and 53.4% in P. vivax (Figure 1). These rates are consistent with prior adult studies, where P. falciparum malaria is strongly associated with anemia through hemolysis and impaired erythropoiesis (41), P.vivax with moderate but common anemia, about 30–40%, in endemic regions (33) and Mixed infections showing variable hematological outcomes (34). The elevated rates in our Mixed malaria cohort likely reflected higher parasite densities (19,32). Importantly, species-based differences did not reach statistical significance, which may reflect sample size limitations, overlapping severities, and standardized hospital care reducing inter-species variation.

Renal complications. falciparum infection is strongly associated with acute kidney injury, reported in 20–40% of hospitalized cases, driven by microvascular sequestration, intravascular hemolysis, and immune-mediated glomerular injury (36,37). In our malaria cohorts, renal

involvement occurred in 16% overall, highest in P. falciparum malaria (40%), followed by Mixed malaria (17.4%) and P. vivax (7.6%). Notably, Mixed infections showed fewer renal complications than P. falciparum alone, suggesting a modulatory role of P. vivax. Evidence indicates that P. vivax elicits balanced immune responses with higher IL-10 and regulatory cytokine activity (19), limiting inflammation and end-organ damage. In addition, its preference for reticulocytes reduces parasite biomass and sequestration in renal microvasculature (20) These mechanisms may explain the lower renal complication rates in mixed malaria, highlighting a potential protective effect of P. vivax in co-infection.

Hepatic injury is a recognized complication of malaria. In this study, it was observed in 72% of P.falciparum cases versus 39% of P. vivax and 41% of Mixed infections. These findings align with international reports of hepatic dysfunction in 40–70% of P.falciparum cases, (39). Histopathology demonstrates hepatocellular apoptosis, sinusoidal congestion, and signaling pathways like NF-κB activation, implicating inflammatory and apoptotic pathways (40). These findings are consistent with the broader pathogenic basis of malaria, where P. falciparum exerts systemic effects through sequestration and immune activation (41). P. vivax, though traditionally less hepatotoxic, causes hepatic dysfunction in 20–40% of cases, especially in relapse-prone populations and those with comorbidities (42,55). Mechanistically, P.vivax injury is immune-mediated but tempered by IL-10 and transforming growth factor beta (TGF-β) activity (19,42,). In our study, hepatic injury was lower in Mixed infections, consistent with earlier reports showing fewer complications in P.falciparum–vivax co-infection. By limiting inflammation and microvascular congestion, P. vivax may counterbalance P. falciparum pathology, mitigating hepatic injury in Mixed malaria (18,19).

Metabolic Acidosis. Metabolic acidosis, a key predictor of malaria morbidity and mortality, was significantly more frequent in P. falciparum (64%) compared to Mixed malaria (39.8%) in this study with an odds ratio of 2.7 (95% CI 1.1–7.5). This underscores P.falciparum's unique pathophysiology, where sequestration of parasitized erythrocytes impairs perfusion and drives lactic acid accumulation (43). In contrast, P. vivax (39.1%) shows less acidosis due to minimal endothelial adhesion (42). Although prior reports suggest Mixed malaria exacerbate acidosis through combined parasite burden and inflammation (5), our malaria cohorts diverged, with lower rates in mixed cases—possibly reflecting attenuation by P.vivax species lacking endothelial adhesion. This shift in pathogenesis is consistent with P. vivax biology, where minimal cytoadherence reduces end-organ dysfunction and complications are more often hematological, including anemia and thrombocytopenia (41,42).

Hyponatremia. was observed in 76% of P. falciparum, 40% of P. vivax, and 60% of Mixed malaria infections. Using mixed as reference, P. vivax showed significantly lower odds (OR 0.5, 95% CI 0.2–0.9), while P.falciparum showed higher odds (OR 1.9, 95% CI 0.4–10.6) ,though the comparison of P. falciparum versus mixed cohorts did not achieve statistical significance. Never the less it's safe to say that hyponatremia was less frequent in vivax and tended to be more common in falciparum cohort. Literature has reported higher hyponatremia in falciparum than vivax, as falciparum is known to cause electrolyte imbalance (49). In contrast, other reports suggest mixed infections exacerbate sodium disturbances via combined parasite burden and inflammation (5). In our malaria cohorts, P. falciparum showed slightly higher rates than mixed. Pathophysiology involves hypovolemia, inappropriate vasopressin release, and impaired renal sodium handling rather than cytoadherence (41,50). P. vivax generally produces milder disturbances due to absent

sequestration (42), while P.falciparum's systemic inflammation predisposes to severe hyponatremia (49). The plausible explanation of lesser hyponatremia in mixed cohort versus falciparum could be that competition to falciparum presented by the presence of vivax may lead to lesser sequestration (5).

Leukopenia. is more often associated with P. vivax thanP. falciparum, largely due to splenic sequestration and immune-mediated marrow suppression (51,5). P.falciparum, in contrast, is less commonly linked and may present with leukocytosis in severe disease, reflecting systemic inflammatory activation (52). Evidence for Mixed malaria infections is inconsistent: some studies report higher leukopenia from additive parasite burden and immune dysregulation (5), while others find no difference compared to mono-infections (53). In our malaria cohorts, leukopenia occurred in 29.6% of vivax, 12.5% of falciparum, and 23.9% of mixed cases (Figure 1), with no significant differences. Nonetheless, the tendency toward greater leukopenia in vivax and mixed infections aligns with studies which report that mixed malaria, through splenic pooling, and immune suppression, predisposes to leukocyte reduction (5,51).

Hospital Course and Outcomes.

The average hospital stay was short (3.1 ± 2.5 days, range 1–20), with medians of 02 days for P. falciparum, 03 P. vivax , and 03 for Mixed malaria infections. Although P. falciparum had the shortest median stay—unexpected given its severity profile—prolonged admissions in our malaria cohorts were driven by persistent thrombocytopenia and leukopenia, which were more frequent in P.vivax and Mixed malaria cohorts (53). This explains slightly longer stays in non-falciparum and mixed cases despite P.falciparum's classical severity. Differences approached significance

346 ($p = 0.053$), suggesting species type may influence admission duration, but hematological recovery
347 was more decisive.
348 One mortality occurred in a 29-year-old with P. vivax malaria complicated by NSTEMI,
349 underscoring those comorbidities and atypical complications can worsen outcomes even in species
350 considered less severe (54). Literature confirms that rare but serious manifestations, including
351 cardiac and metabolic involvement, may occur in non-falciparum malaria (51,54). Overall,
352 hospital stays were brief, but vigilance is essential for hematological complications.
353 **Impact of Sociodemographic and Comorbid Conditions.**
354 It is important to acknowledge that hypertension, diabetes mellitus, ischemic heart disease, age,
355 and gender are well-established determinants of outcomes such as acute kidney injury, metabolic
356 acidosis, anemia, and other organ dysfunctions. In our study, these factors were not balanced
357 between groups, and therefore the associations observed should be interpreted as exploratory rather
358 than causal. Post hoc analyses nevertheless revealed significant sociodemographic influences:
359 older age (>40 years) and female sex were associated with anemia, consistent with prior literature
360 attributing this to iron deficits from menstruation, pregnancy, and dietary inequities (35). Acute
361 kidney injury was strongly linked to hypertension, diabetes, and ischemic heart disease in line with
362 prior studies highlighting the impact of comorbidities on malaria outcomes (36,37). Similarly,
363 metabolic acidosis was more frequent in older patients (>40 years, reflecting reduced physiological
364 reserve and impaired lactate clearance (44). Hypertension increased acidosis risk, likely due to
365 endothelial damage and reduced vascular compliance compounded by falciparum sequestration
366 (45), while diabetes further amplified risk, consistent with impaired glucose metabolism,
367 susceptibility to lactic acidosis, and reduced renal clearance (38). Interestingly, acidosis was less

frequent in men, despite reports of higher parasite densities in males (46). This paradox may reflect sex-linked differences in immune regulation and metabolic reserve. Most affected women were premenopausal (25–41years), suggesting hormonal influences; estrogen and progesterone modulate immune responses and vascular tone, with estrogen enhancing pro-inflammatory cytokines (TNF-α, IL-6), thereby amplifying systemic inflammation and lactate accumulation (47,48). Older patients (>40 years) in our study were about twice as likely to develop hyponatremia, consistent with age being a recognized risk factor for electrolyte disturbances (35,44). However, Collectively, these complications underscore the complex interplay between malaria and non-communicable diseases. While outside the original scope of this paper, they highlight the need for adequately powered, prospective studies specifically designed to evaluate the impact of comorbidities and sociodemographic factors on malaria-related organ dysfunction across species.

Study Limitations

This retrospective, single-center design relied on clinical parameters without immunological assays, so mechanisms remain speculative and generalizability limited. Age, gender, and comorbidities were not balanced across cohorts, restricting causal inference and potentially influencing associations with anemia, metabolic acidosis, and acute kidney injury. Diagnosis based on ICT kits and peripheral smears may have under-detected mixed infections compared to PCR. Referral bias likely enriched the dataset with severe cases, while small subgroup sizes—particularly P. falciparum—reduced statistical power. Larger multicenter studies using molecular diagnostics and immunological profiling are needed to validate these findings.

Conclusion:

Our study demonstrates that mixed P.falciparum–P. vivax infections were associated with comparatively reduced severity of organ dysfunction compared to mono-species infections. P. falciparum mono infection carried the highest risk of renal, hepatic, and metabolic complications, while P. vivax mono infection was strongly linked to thrombocytopenia. Mixed infections showed a relative reduction in severity, suggesting possible modulatory interactions between species. Outcomes were also influenced by host factors such as age, sex, and comorbidities (hypertension, diabetes mellitus, ischemic heart disease), though these were not balanced across the various malaria cohorts and should be interpreted cautiously. Despite the limitations of a retrospective design, our findings provide novel evidence that mixed infections may mitigate disease severity, challenging assumptions of uniformly enhanced morbidity. These insights have practical implications for clinical triage, ward allocation, and resource utilization in endemic regions, and highlight the need for prospective multicenter studies.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

List of Abbreviations

AKI: Acute Kidney Injury

ALT: Alanine Aminotransferase

AST: Aspartate Aminotransferase

CI: Confidence Interval

G6PD: Glucose-6-Phosphate Dehydrogenase

GCS: Glasgow Coma Scale

HLA: Human Leukocyte Antigen

ICT: Immunochromatographic Test

IFN-γ: Interferon gamma

IL-10: Interleukin-10

IL-6: Interleukin-6

IQR: Interquartile Range

LDH: Lactate Dehydrogenase

MP: Malarial Parasite

NF-κB: Nuclear Factor kappa-light-chain-enhancer of activated B cells

NSTEMI: Non-ST Elevation Myocardial Infarction

OR: Odds Ratio

PCR: Polymerase Chain Reaction

RDT: Rapid Diagnostic Test

RR: Respiratory Rate

SpO_2: Peripheral Oxygen Saturation

SPSS: Statistical Package for the Social Sciences

TGF-β: Transforming Growth Factor beta

TNF-α: Tumor Necrosis Factor alpha

Tregs: Regulatory T cells ($CD4^+CD25^+FoxP3^+$)

WHO: World Health Organization

γδ T cells: Gamma-delta T cells

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Authors' contribution

AHZ conceptualized the study. AHZ, SNZ, AJ designed the study protocol. IFA, HIK, HS, IBA collected and entered the data. AHZ, SNZ, AJ, IFA, HIK, HS, IBA drafted the manuscript. SNZ critically reviewed and revised the initial draft. All authors read and approved the manuscript.

ETHICS DECLARATION

Ethics Approval Statement

The study was approved from Ethics Committee of Liaquat National Hospital (APP # 1248-2025-LNH-ERC). Informed consent was waived due to the retrospective design. Confidentiality and

anonymity were maintained throughout the research. The study was performed following the guidelines of Declaration of Helsinki (2013).

Consent for publication

Not applicable

Competing Interests

The authors declare no competing interests.

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The authors acknowledge that artificial intelligence (AI) assistance was used to support manuscript refinement, including language polishing, keyword optimization, and cover-letter drafting. All scientific content, data analysis, and interpretations remain the responsibility of the authors.

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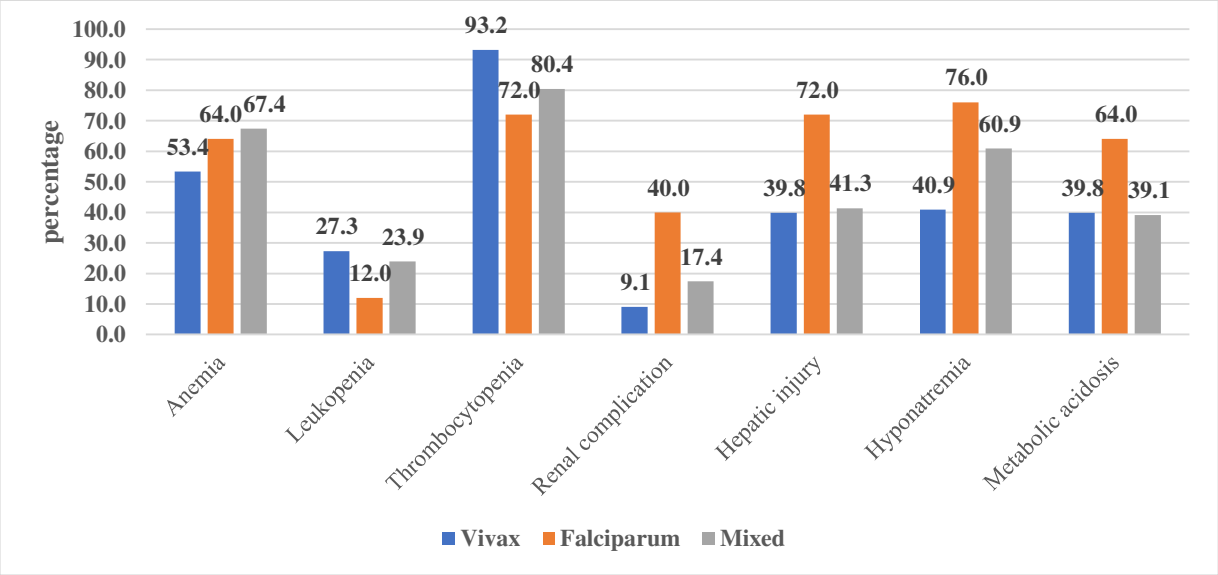


Figure 1: A comparative analysis of complications observed across various categories of cases.

Table 1: Vital signs at presentation across malaria cohorts

Variables	**Non-falciparum**	**Falciparum**	**Mixed**
Systolic	114.1±13.4	111.7±10.4	117.3±10.6
Diastolic	73.3±11.1	75.0±5.0	75.9±8.5
Pulse	84.4±15.7	83.0±8.2	82.7±19.2
Temperature	97.8±0.8	98.0±0.0	97.9±0.4
RR	18.5±1.7	17.0±3.0	18.8±2.1
SpO2	97.6±1.5	98.0±0.0	97.9±1.0

Table 2: Patients' sociodemographic and clinical features among different malaria cohorts

Variables	Sub-groups	Non-Falciparum	Falciparum	Mixed	p-value
Age	<40 years	47(53.4)	17(68)	22(47.8)	0.260
	≥40 years	41(46.6)	8(32)	25(52.2)	
Gender	Male	57(64.8)	16(64)	31(67.4)	0.943
	Female	31(35.2)	9(36)	15(32.6)	
Occupational settings	Outside	3843.2)	13(52)	22(47.8)	0.703
	Inside	50(56.8)	12(48)	24(52.2)	
Presenting features	Fever	85(96.6)	23(92)	43(93.5)	0.417
	Cough	7(8)	4(16)	8(17.4)	0.221
	Vomiting	29(33)	6(24)	15(32.6)	0.682
	Epigastric pain	6(6.8)	4(16)	2(4.3)	0.203
	Generalized weakness	19(21.6)	6(24)	9(19.6)	0.907
	Shortness of breath	9(10.2)	3(12)	7(15.2)	0.699
	Headache	5(5.7)	2(8)	5(10.9)	0.542
	Body ache	24(27.3)	6(24)	13(28.3)	0.926
	Loose stools	12(11.4)	1(12.5)	7(15.2)	0.607

Table 3: Univariate association of complications with Malaria diagnosis

Complications	**Vivax** **OR (95% CI)**	**Falciparum** **OR (95% CI)**	**Mixed Malaria**
Anemia	0.6 (0.3 – 1.2)	0.8 (0.2 – 3.8)	Ref category
Leukopenia	1.3 (0.6-2.9)	0.5 (0.1 - 4.1)	Ref category
Thrombocytopenia	*3.3 (1.1 – 10.0)	0.6 (0.2 – 1.9)	Ref category
Renal complication	0.5 (0.2 - 1.4)	*3.2 (1.1 - 9.5)	Ref category
Hepatic injury	0.9 (0.4 - 1.9)	*3.7 (1.3 – 10.5)	Ref category
Hyponatremia	*0.4 (0.2 - 0.9)	2.09 (0.6 – 6.1)	Ref category
Metabolic acidosis	1.0 (0.5 - 2.1)	*2.7 (1.1 - 7.5)	Ref category

*Significant at $p<0.05$, **Significant at $p<0.001$

Table 4: Association of complications with Comorbid Conditions

Complications	>40 years	Male	Hypertension	Diabetes	IHD
Anemia	*2.1(1.1-4.1)	*0.1(0.04-0.2)	2(0.9-4.1)	1.6(0.7-3.6)	1.7(0.4-6.7)
Hemolytic anemia	1.3 (0.6 - 2.5)	0.6 (0.3 - 1.2)	1.4 (0.7 - 3.0)	1.7 (0.7 - 3.8)	2.9 (0.8 - 10.6)
Leukopenia	0.5 (0.3 - 1.0)	0.7 (0.3 - 1.3)	0.6 (0.3 - 1.5)	0.5 (0.2 - 1.3)	1.2 (0.3 - 4.7)
Thrombocytopenia	0.7 (0.3–2.0)	0.9 (0.3–2.7)	0.6 (0.2–1.7)	0.6 (0.2–1.8)	0.5 (0.1–2.5)
Acute kidney injury	2.4 (0.8–7.2)	1.1 (0.4–3.0)	**6.1 (2.1–17.3)	**7.1 (2.5–20.2)	**6.4 (1.6–25.5)
Hepatic injury	0.8 (0.4 - 1.5)	1.3 (0.7 - 2.6)	0.8 (0.4 - 1.5)	0.6 (0.3 - 1.5)	1.0 (0.3 - 3.6)
Hyponatremia	*1.9(1.0 - 3.6)	1.6(0.8 - 3.1)	1(0.5 - 1.9)	0.9(0.4 - 2.1)	1(0.3 - 3.6)
Metabolic acidosis	*2.39 (1.25 - 4.57)	* 0.5 (0.2 - 0.9)	*2.3 (1.2-4.7)	*3.0 (1.3-6.9)	3.4 (0.8-13.5)

*Significant at $p<0.05$, **Significant at $p<0.001$. Values are odds ratios (95% confidence interval).

IHD, ischemic heart disease.