ORIGINAL ARTICLE

A Cross-Sectional Study of Malaria and Dengue Co-Infection in a Tertiary Care Hospital in Balochistan

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ABSTRACT

Objective: To determine the frequency and clinical profile of malaria–dengue co-infection among febrile patients in Quetta, Pakistan.

Study Design: A cross-sectional observational study.

Place and Duration of Study: The study was conducted at the Department of Hematology, Combined Military Hospital, Quetta (Balochistan), Pakistan, from May 2025 to October 2025.

Methods: A total of 355 patients aged ≥12 years presenting with acute febrile illness were evaluated. Malaria was diagnosed using thick and thin smears on microscopy. Serological tests (IgM and NS1) for dengue were performed for confirmation. Hematological parameters were analyzed on a Sysmex XN-350 hematology analyzer. Data was analyzed by using Statistical Package for Social Sciences (SPSS) 26.00, where the Chi-Square test was used for categorical variables, while the Mann–Whitney U test and the Kruskal–Wallis test were applied for comparison of non-normally distributed quantitative variables, with $P \leq 0.05$ considered statistically significant.

Results: Malaria was confirmed in 282 (79.4%) cases, dengue in 27 (7.6%), and dual infection in 15 (4.2%), predominantly Plasmodium vivax (73%). The highest proportion of affected individuals (55.6%) fell in the 26–50-year age group. Fever and myalgia (87.5%) were the most frequent symptoms in co-infected cases. Platelet counts were lowest in co-infection [100.00 (108.50 – 80.00) × 10^9/L, $P < 0.001$, while hemoglobin was significantly reduced in malaria patients ($P = 0.271$). Dengue + P. falciparum co-infection showed greater bleeding tendency and one fatal outcome due to dengue hemorrhagic fever.

Conclusion: Dengue–malaria co-infection, although relatively uncommon, presents with overlapping clinical manifestations. This can even result in severe thrombocytopenia. Routine dual testing for malaria and dengue in febrile illnesses can facilitate early diagnosis and improve patient outcomes in endemic areas.

Keywords*: Co-Infection, Dengue, Malaria, Pakistan, Thrombocytopenia.*

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Introduction

Malaria and dengue are significant contributors to disease burden in endemic areas, including Southeast Asia. Although two entirely different species of mosquitoes, Anopheles for malaria and Aedes aegypti for dengue, are responsible for their spread, both infections often occur in the same areas. This overlap is a major health concern in South Asia, especially in Pakistan.[1]

Plasmodium, an obligate parasite, is the cause of malaria in humans. Of the five species that infect humans, P. vivax and P. falciparum are most common in Pakistan. Infection begins when an infected Anopheles mosquito bites a person, leading to fever, chills, and anaemia. Failure to recognize severe disease can lead to multi-organ failure.[1] National data show malaria affects around 23.3% of the population, with P. vivax responsible for nearly 80% of infections.[1] Transmission usually increases after the flood season, when stagnant water allows

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mosquitoes to breed.[2]

Conversely, Dengue is a viral illness caused by four dengue virus serotypes (DEN-1 to DEN-4). Spread is through Aedes aegypti, which breeds in clean, still water. The patient presents with high fever, body aches, headache, and rash, which may lead to bleeding, plasma leakage, or shock. Typical laboratory findings include low platelet and white cell counts.[2,3] There has been an increase in Dengue outbreaks in Pakistan targeting urban areas like Karachi, Lahore, and Rawalpindi.[2] Poor sanitation, rapid urban growth, and weak mosquito control programs worsen the problem.[4] Misdiagnosis is common because dengue and malaria share many symptoms (fever, myalgia, and low platelets). In addition, illness can become more severe and unpredictable when both infections occur simultaneously.[5,6] Studies from Pakistan have reported such dual infections, highlighting the need to test for both diseases in febrile patients.[2,7]

This issue is particularly relevant in Balochistan. The region's dry climate, irrigation practices, and poor drainage encourage mosquito breeding. Seasonal rains and population movement add to the spread, while rural diagnostic facilities are limited. Repeated post-monsoon fever outbreaks in Quetta and nearby districts suggest ongoing transmission of both malaria and dengue.[1] However, little is known about the extent of their co-infection in this area. Early detection is crucial since both diseases can quickly become severe. Therefore, this study aimed to determine the frequency and clinical features of malaria–dengue co-infection among patients presenting with acute fever in a tertiary hospital in Quetta. The findings will not only help clinicians with the early detection of co-infection but also assist in allocating resources for improved prevention and vector control efforts in malaria-endemic areas of Pakistan.

Methods

The research was conducted from May 2025 to October 2025, as a single-center, cross-sectional observational study at the Departments of Hematology, Combined Military Hospital (CMH), Quetta (Balochistan), Pakistan. The study aimed to determine the frequency of malaria and dengue co-infection in patients presenting with acute febrile illness at a tertiary care hospital. The WHO sample size calculator was used to determine the sample size, using an expected infection rate of 27%, a 95% confidence interval, and a 5% margin of error. Although 303 participants were required, 355 consecutive patients were included to enhance study precision.[8]

A total of 355 patients presenting with acute febrile illness were included in the study. Patients aged 12 years or older of both genders, presenting with fever of suspected malarial or dengue origin, were enrolled using non-probability consecutive sampling. Patients with other confirmed infections (e.g., typhoid, sepsis) or who had received prior antimalarial therapy were excluded. Ethical approval was obtained from the Institutional Review Committee of the hospital, vide certificate no: CMH QTA-IERB/75/2024, dated: 11th February 2024. Written informed consent was taken from all participants. The study adhered to the Declaration of Helsinki.

Approximately 5 mL of venous blood was collected in EDTA tubes for complete blood counts and malaria testing. Thick and thin blood smears were prepared—thick smears for parasite detection and thin smears for species identification (P. vivax or P. falciparum). An additional 2 mL sample was collected in a plain tube for dengue serology (IgM antibody and NS1 antigen detection). Hematological parameters, including hemoglobin, platelet count, and total leukocyte count, were analyzed using a Sysmex XN-300 hematology analyzer (Sysmex Corporation, Kobe, Japan). All laboratory analyses were performed in the same facility, ensuring strict internal quality control. Hematological and biochemical parameters between patients with dengue/vivax and dengue/falciparum co-infection were observed.

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 26.00. Normality of data was checked by using the Kolmogorov-Smirnov test. Quantitative variables age, hemoglobin, platelet count, alanine aminotransferase (ALT), and Total leucocyte count (TLC) were non-normally distributed and represented by median (IQR). Qualitative data was represented using percentages and frequencies. Chi-square test (for qualitative variables), for quantitative data, the Mann-Whitney U test (for two groups), and the

Kruskal-Wallis Test (for three groups were applied. A *P*-value of ≤ 0.05 was considered as statistically significant.

Results

A total of 355 patients presenting with acute febrile illness were included. Out of which 274 (77.2%) patients were males, and 81 (22.8%) patients were females. Median age was 30 years (IQR, 24-36). Based on initial evaluation, 339 (95.5%) were clinically suspected of malaria, and 16 (4.4%) of dengue fever. However, laboratory confirmation identified malaria in 282 patients (79.4%) using thick and thin blood smears and dengue infection in 27 patients (7.6%) through positive dengue serology (IgM antibody and NS1 antigen detection). Dual infection with malaria and dengue was detected in 15 cases (4.2%), predominantly Plasmodium vivax (11 cases, 40.7%), followed by P. falciparum (4 cases, 14.8%). The highest proportion of affected individuals (55.6%) fell in the 26–50-year age group, suggesting greater vulnerability among young and middle-aged adults.

Table 1 compares the clinical features and laboratory parameters among patients with dengue, malaria, and dengue–malaria co-infection. Fever was present in all dengue and co-infected patients and in the majority of malaria cases; however, the difference was not statistically significant (P = 0.067). Abdominal pain and nausea were also observed across the groups, with no significant differences (P = 0.052 and P = 0.146, respectively). In contrast, several symptoms were significantly more frequent in co-infected patients compared with those having malaria alone, including headache (43.8% vs. 14.7%, P = 0.007), vomiting (62.5% vs. 28.6%, P = 0.016), joint pain (43.8% vs. 14.3%, P = 0.005), rash (56.3% vs. 9.4%, P < 0.001), myalgia (87.5% vs. 19.9%, P < 0.001), bleeding manifestations (18.8% vs. 3.0%, P = 0.001), and jaundice (25.0% vs. 7.5%, P = 0.001). Regarding laboratory parameters, platelet counts were significantly lower in co-infected patients [median 100 × 10^9/L (IQR 80.00–108.50)] than in the other groups (P < 0.001). However, hemoglobin levels, total leukocyte count, and alanine aminotransferase (ALT) did not differ significantly among the three groups (P = 0.271, P = 0.769, and P = 0.122, respectively).

Table 2 presents the detailed comparison of

Table 1: Clinical features of dengue, malaria, and co-infected patients (N=293)

Clinical Feature	Dengue (N=11) Frequency (%)	Malaria (N=266) Frequency (%)	Co-infection (N=16)	*Test* value	*P*-value
Fever	11 (100.0%)	221 (83.1%)	16 (100.0%)	χ2=5.396	0.067
Abdominal pain	2 (18.2%)	142 (53.4%)	10 (62.5%)	χ2=5.920	0.052
Nausea	4 (36.4%)	101 (38.0%)	10 (62.5%)	χ2=3.849	0.146
Headache	7 (63.6%)	39 (14.7%)	7 (43.8%)	χ2=9.892	0.007ǂ
Vomiting	3 (27.3%)	76 (28.6%)	10 (62.5%)	χ2=8.267	0.016ǂ
Joint pain	3 (27.3%)	38 (14.3%)	7 (43.8%)	χ2=10.554	0.005ǂ
Rash	4 (36.4%)	25 (9.4%)	9 (56.3%)	χ2=34.892	< 0.001ǂ
Myalgia	7 (63.6%)	53 (19.9%)	14 (87.5%)	χ2=45.426	< 0.001ǂ
Bleeding	2 (18.2%)	8 (3.0%)	3 (18.8%)	χ2=13.914	0.001ǂ
Jaundice	4 (36.4%)	20 (7.5%)	4 (25.0%)	χ2=14.839	0.001ǂ
Haemoglobin- Hb (g/dl)*	13.40 (15.5 – 12.9)	12.50 (13.70 – 11.28)	12.70 (13.93 – 12.00)	KW=2.614	0.271ǂ
Count -TLC (x109/L) * Platelet count- Plt (x109/L)*	7.300 (16.0 – 3.20)	5.90 (7.70 – 4.87)	7.20 (9.58 – 4.15)	KW=0.769	0.769
Alanine Transferase -	226.00 (281.0 – 100.0)	187.50 (315.50 – 141.50)	100.00 (108.50 – 80.00)	KW=27.183	<0.001ǂ
ALT (u/l)*	31.00 (35.0 – 24.0)	33.00 (39.00 – 29.00)	34.00 (46.75 – 30.00)	KW=4.202	0.122

**Median (IQR), KW= Kruskal Wallis Test, χ2=Chi-Square, ǂ Statistically significant values (P ≤ 0.05)*

Table 2: Laboratory parameters of dengue/Vivax & dengue/falciparum coinfection (N=15)

Parameter	Dengue/Vivax (N=11) (Median ± IQR)	Dengue/Falciparum (N=4) (Median ± IQR)	*Test* value	*P*-value
Hemoglobin (g/dL)	12.80 (13.40 – 12.00)	14 (16.68 – 10.88)	U=17.00	0.513
Platelet count ($\times10^9$/L)	100 (100.00– 80.00)	100.50 (223.25 – 49)	U=20.50	0.842
ALT (U/L)	31.00 (47.00– 30.00)	40 (55.75 – 34.00)	U=13.00	0.238
TLC ($\times10^9$/L)	4.80 (9.50 – 3.80)	9.30 (9.95 – 7.45)	U=9.50	0.102

U = Mann-Whitney U test

hematological and biochemical parameters between patients with dengue/vivax (N = 11) and dengue/ falciparum (N = 4) co-infection. The median hemoglobin level was slightly higher in the dengue/ falciparum group [14 (16.68–10.88) g/dl] compared to the dengue/vivax group [12.80 (13.40–12.0) g/dl]; however, this difference was not statistically significant (*P* = 0.513). However, Platelet counts were similarly reduced in both groups, with median values of 100 (100–80) $\times10^9$/L in dengue/vivax and 100.50 (223.25–49) $\times10^9$/L in dengue/falciparum co-infection, with no statistically significant difference (*P* = 0.842). Liver involvement, assessed by alanine aminotransferase (ALT), was modestly higher in the dengue/falciparum group [40.0 (55.75–34.0) U/L] than in the dengue/vivax group [31.0 (47.0–30.0) U/L], though this variation was also not statistically significant (*P* = 0.238). Moreover, Total leucocyte count (TLC) tended to be higher in patients with dengue/falciparum co-infection [9.30 (9.95–7.45) $\times10^9$/L] than in those with dengue/vivax co-infection [4.80 (9.50–3.80) $\times10^9$/L]; however, the difference did not reach statistical significance (*P* = 0.102).

Discussion

This cross-sectional study was carried out to evaluate the frequency, clinical features, and hematological parameters of malaria, dengue, and their co-infection. Around 355 patients presented with acute febrile illness at the Combined Military Hospital Quetta, Balochistan, and participated in the study. Malaria was confirmed in 79.4% of cases, dengue infection in 7.6%, and malaria–dengue co-infection in 4.2%, respectively. The majority of co-infected cases (N=11) were Plasmodium vivax, compared to only 4 cases of Plasmodium Falciparum. These findings indicate that although co-infection is relatively uncommon, it remains a clinically relevant entity in endemic regions where both infections coexist.

The finding of dengue –malaria coinfection observed in our study (4.2%) is consistent with previous local studies in Pakistan. Siddique S et al. reported a similar co-infection rate of approximately 4.2% among febrile patients admitted to a tertiary care hospital in Lahore.[2] Likewise, comparable rates were observed in DI Khan and Peshawar studies, with co-infection frequencies of 3.8% to 5.1% among febrile admissions, with P. vivax as the dominant species.[9,10] These findings suggest that dual infection is not rare in malaria-endemic regions of Pakistan. This may remain underdiagnosed if patients are evaluated for only one pathogen during acute febrile illness.

In terms of demographic characteristics, the largest proportion of affected patients in our study was in the 26–50-year age group (55.6%). This age distribution likely reflects greater occupational exposure and outdoor activity among young and middle-aged adults. This also increases the risk of mosquito bites, making them more vulnerable. Similar age distributions have been reported in previous regional studies of malaria and dengue infections in Pakistan, where the majority of affected patients belonged to the economically active age group.[11,12] Male predominance was also observed in our cohort, as reported in several regional studies, and may be attributed to increased environmental exposure among males in endemic areas.[13-17]

Regarding clinical manifestations, several symptoms were significantly more frequent in patients with dengue–malaria co-infection compared with malaria alone. In our study, Fever was present in the majority of patients across all three groups (83-100% of the cases) and did not demonstrate a statistically significant difference (*P* = 0.067). This finding emphasizes that fever alone cannot reliably distinguish between malaria, dengue, and their co-infection, as it represents a common presenting feature of both diseases. Moreover, headache, vomiting, joint pain, rash, myalgia, bleeding manifestations, and jaundice showed statistically significant differences between the groups (*P* = 0.007, *P* = 0.016, *P* = 0.005, *P* < 0.001, *P* < 0.001, *P* =

0.001, *P* = 0.001*,* respectively). Among these, myalgia was particularly prominent in co-infected patients (87.5% vs. 19.9% vs 63.6%) than malaria and dengue alone. Similarly, headache was seen more in dengue, whereas vomiting was seen in malaria-infected patients. Our co-infected patients also exhibited some features seen in malaria (like gastrointestinal symptoms), rendering diagnosis confusing and time-consuming. Symptom clustering was reported in regional Pakistani, Thai, and African studies, also, where dengue features dominated co-infection presentations.[2,6,13-16] Such variability may relate to local mosquito ecology and circulating viral serotypes. This may raise concerns about missed or delayed diagnosis of dengue cases in malaria-endemic settings.[3]

The combined effects of dengue-associated platelet destruction and malaria-related splenic sequestration may contribute to the more pronounced thrombocytopenia observed in co-infected patients.[7,18] Analysis of laboratory parameters of our groups demonstrated that thrombocytopenia was the most significant hematological abnormality associated with co-infection (*P* <0.001). Platelet counts were significantly lower in co-infected patients compared with patients with malaria or dengue alone [median 100.00 $\times 10^9$/L (IQR 80.00–108.50)]. This observation is consistent with previous studies that have identified severe thrombocytopenia as a key laboratory finding in dengue and dengue-related co-infections.[7,9,10] Unlike the studies from Lahore and Peshawar, which reported both thrombocytopenia and anemia as the most frequent abnormalities.[2,9] Kotepui M et al. in their meta-analysis also reinforced that co-infection magnifies hematologic stress, producing compounded thrombocytopenia and anemia.[5] However, our findings showed statistically significantly reduced platelet counts (*P* < 0.001) and non- significant hemoglobin values (*P* = 0.271).

Mild hepatic involvement, seen as elevated ALT in our study (34.00 (46.75 – 30.00) U/L, *P* = 0.122), was also documented by Alsedig K et al. in Sudan and Naing C et al. in Southeast Asia.[6,19] Both reported raised transaminases due to overlapping inflammatory and immune responses, supporting our observation of liver stress in co-infected patients. However, the non-significant findings (*P* = 0.271*, P* = 0.122) among the three groups in our study suggest that although anemia and hepatic involvement may occur in malaria and dengue infections, these parameters may not reliably differentiate co-infection from mono-infection in clinical practice.

Further comparison between dengue/P. vivax and dengue/P. Falciparum co-infection in our study showed no statistically significant differences in hemoglobin levels, platelet counts, total leukocyte count, or alanine aminotransferase levels. However, a trend toward higher hemoglobin levels and leukocyte counts was observed in dengue/P. falciparum co-infection. Previous studies have suggested that P. falciparum infection may be associated with more severe clinical manifestations and greater hematological disturbances compared with P. vivax infection.[2,20,21] In our cohort, one fatal outcome was observed in a patient with dengue and P. falciparum co-infection who developed dengue hemorrhagic fever, which may reflect the potentially severe clinical course associated with this combination. Uneventful recovery in most dengue + P. vivax cases in our study was consistent with findings from Peshawar and DI Khan, where P. vivax co-infection rarely caused severe disease.[9,10] This difference may reflect distinct immunopathological interactions between the dengue virus and Plasmodium species.

This study has several limitations. First, the number of co-infected patients was relatively small, which limited the statistical power for subgroup analysis. Second, dengue diagnosis was based on serological testing rather than molecular confirmation, which may lead to cross-reactivity and overestimation of dengue positivity. Third, the study was conducted at a single tertiary care center, which may limit the generalizability of the findings to other regions.

Despite these limitations, this study provides important data on the frequency and clinical characteristics of malaria–dengue co-infection in Balochistan, a region where epidemiological data on this topic remain limited. Uniform diagnostic criteria and rigorous statistical comparison further added strength to this study. The results highlight the need for heightened clinical awareness and routine diagnostic evaluation for both infections in patients presenting with acute febrile illness in endemic areas.

Conclusion

Dengue–malaria co-infection, though uncommon, poses diagnostic and therapeutic challenges in endemic regions. Our findings confirm significant hematologic and hepatic alterations, with dengue + falciparum infection showing a higher risk of hemorrhagic outcomes. Early recognition through combined screening and vigilant monitoring can prevent complications. Larger multicenter studies are warranted to further clarify disease interactions and guide targeted interventions.

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Author Contributions

NA: Conception, design of the work, manuscript writing for methodology design, investigation, validation of data, interpretation, write-up of results, and approval for final submission

NKA: Writing the original draft, proofreading, and approval for final submission

SK: Data acquisition, curation, statistical analysis, and approval for final submission

MZ: Revising, editing, supervising for intellectual content, and approving for final submission

NA is the nominated guarantor and takes full responsibility for the overall content and integrity of the work

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