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Dengue–Malaria Co-infection and Circulating Dengue Serotypes: A Retrospective Study from a Tertiary Care Centre in Western India

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ABSTRACT

Background: Dengue and malaria are major vector-borne diseases with overlapping geographic distribution and clinical presentation. Co-infection poses diagnostic and therapeutic challenges. To determine the pattern of dengue–malaria co-infection and identify circulating dengue serotypes among co-infected patients.

Methods: A retrospective study was conducted in the Department of Microbiology, Government Medical College, Surat, from January 2021 to December 2025. Dengue-positive cases (NS1 antigen and/or IgM ELISA) were traced for malaria diagnosis using peripheral blood smear. Co-infected cases were further analyzed for dengue serotypes where available.

Results: A total of 20 dengue–malaria co-infected cases were identified. The age ranged from 4 to 60 years, with a predominance of males. Most cases occurred during the monsoon/post-monsoon period, particularly in August. *Plasmodium vivax* was more frequently observed than *P. falciparum*. Dengue serotyping (available for a subset) revealed circulation of DEN-1, DEN-2, and DEN-3, with DEN-2 and DEN-3 being common.

Conclusion: Dengue–malaria co-infection, though relatively uncommon, demonstrates a clear seasonal trend and involves multiple dengue serotypes. Routine screening for both infections during peak transmission periods is essential to ensure timely diagnosis and management.

Key-words: Dengue, Malaria, Co-infection, Dengue serotype, Vector-borne diseases

INTRODUCTION

Dengue and malaria are two of the most important vector-borne diseases in tropical and subtropical regions, contributing significantly to global morbidity and mortality [1,2]. Dengue, caused by four antigenically distinct serotypes of dengue virus (DENV-1 to DENV-4) and transmitted by Aedes mosquitoes, has emerged as the most rapidly spreading arboviral infection worldwide [3]. Malaria, primarily caused by *P. falciparum* and *P. vivax* and transmitted by Anopheles mosquitoes, continues to be a major public health problem in endemic regions [4]. The geographical distribution of Aedes and Anopheles vectors overlaps in many parts of Asia, Africa, and Latin America, creating conditions favorable for concurrent infections [5]. Co-infection with dengue and malaria, although considered uncommon, is increasingly reported due to overlapping transmission seasons, climatic changes, and improved diagnostic capabilities [6]. Clinical manifestations of dengue and malaria often overlap—such as acute febrile illness, thrombocytopenia, hepatosplenomegaly, and altered liver function—posing diagnostic challenges, especially in resource-limited settings [7]. Misdiagnosis or delayed recognition of co-infection may result in inappropriate management and increased risk of severe disease and complications [8]. Understanding the epidemiology, clinical profile, and outcomes of dengue–malaria co-infection is crucial for

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guiding patient management and public health strategies. This manuscript aims to highlight the significance of co-infection, discuss its diagnostic and therapeutic challenges, and emphasize the need for integrated vector control and surveillance measures in endemic areas.

Reports from India over the past five years indicate that dengue–malaria co-infection, though relatively uncommon, is increasingly recognized due to overlapping vector distributions and seasonal transmission. In a multicentre pediatric cohort, 20 of 623 children with laboratory-confirmed dengue were found to have malaria co-infection (3.3%) [9]. Similarly, a study from Angul district, Odisha, reported a prevalence of 3.0% among suspected dengue cases tested for both pathogens [10]. More recent analyses of malaria cohorts suggest higher proportions of dengue among patients with any co-infection, with rates approaching 20–25% in selected hospital samples, although these estimates are not population-based [11,12]. Despite variations in diagnostic methods and study designs, available data consistently highlight that dengue–malaria co-infection in India usually occurs at low single-digit percentages in routine hospital or outbreak series, underscoring the need for enhanced surveillance and systematic testing in acute febrile illness.

Dengue virus belongs to the Flaviviridae family and exists as four antigenically distinct serotypes, DENV-1, DENV-2, DENV-3, and DENV-4 [3]. Infection with one serotype usually provides lifelong immunity against the same serotype but only transient and partial protection against the others [2]. Subsequent infection with a heterologous serotype has been associated with increased risk of severe disease due to antibody-dependent enhancement (ADE), which amplifies viral replication and immune-mediated pathology [13].

The role of serotype distribution becomes particularly important in the context of dengue–malaria co-infection. The severity of clinical illness may vary depending on the circulating dengue serotype. Studies have shown that DENV-2 and DENV-3 are often associated with more severe manifestations, including dengue hemorrhagic fever and shock syndrome [14]. When malaria co-exists, overlapping clinical features such as fever, thrombocytopenia, hepatomegaly, and anemia may obscure serotype-specific disease severity, leading to diagnostic delays. Furthermore, co-infection with malaria parasites (*Plasmodium falciparum* or *P. vivax*) may exacerbate immune activation triggered by certain dengue serotypes, increasing the risk of complications such as bleeding, liver dysfunction, or multi-organ involvement [5].

From a public health perspective, understanding circulating dengue serotypes in regions where malaria is endemic is crucial. It not only helps predict the likelihood of severe dengue outcomes but also aids clinicians in managing co-infected patients, where both pathogens may synergistically worsen prognosis. Continuous serotype surveillance, particularly in co-endemic areas of India, is therefore vital to guide patient care and anticipate outbreaks of severe disease in the setting of co-infection [15]. The present study aimed to determine the dengue co-infection rate and identify the dengue serotype in co-infected patients.

MATERIALS AND METHODS

Study design and setting- A retrospective observational study was conducted in the Department of Microbiology, Government Medical College, Surat, from January 2021 to December 2025.

Inclusion Criteria- Samples which were received in Department of Microbiology for Dengue testing either by NS1 or IgM Capture ELISA test and which are positive for any of the test were enrolled in the study.

Exclusion criteria- Samples which were negative for Dengue testing either by NS1 or IgM Capture ELISA test were excluded.

Methodology- Enrolled positive samples were further traced on Laboratory Information Portal system of Hospital for reports on malaria diagnosis by peripheral Blood smear test, which is routinely done in majority of patients along with Complete Blood count by Pathology Department. Permission was taken from Medical Superintendent and Dean of the institute was taken to view the data and to enrol that data for the study. For samples which were positive for either NS1/IgM Capture ELISA for the Dengue and found positive for any malaria species in peripheral blood smear test were further traced for Dengue serotyping results in VRDL section of Microbiology Department. Dengue serotyping was done VRDL by the kits provided by ICMR- NIV, Pune as a part of designated sentinel surveillance program during 2023-

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2024. So we have serotyping data available only for these 2 years for co-infected patients. One more limitation is we have not done all samples analysis which was positive for malaria by peripheral blood smear by pathology Department for their Dengue ELISA results on LIS portal of hospital, only we have traced dengue positive samples from Microbiology Department for their Malaria.

Statistical Analysis- Data were entered in Microsoft Excel and analyzed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Categorical variables were expressed as frequency and percentage, while continuous variables were presented as mean ± standard deviation (SD) or median (interquartile range) as appropriate. Associations between categorical variables were assessed using the Chi-square test or Fisher's exact test. A p-value <0.05 was considered statistically significant.

Ethical Approval- Study was ethically approved by Research and Review Committee of Institute (GMCS/STU/RRC-1/Approval/22607/25, Dated on 14.10.2025). Permission to access laboratory data was obtained from the Medical Superintendent and Dean.

RESULTS

From 2021 to 2025, every year we have approximately 9000-10000 samples for dengue testing comes to Microbiology Department, testing wise and positivity rate are as shown in Table 1.

Table 1: Year-wise Dengue Testing by Dengue NS1 and IgM capture ELISA test and Positivity Rates

Year	Total tested for Dengue IGM	Positive for IgM (Number)	Percentage positivity	Total tested for Dengue NS1	Positive for NS1(Number)	Percentage positivity
2025	5605	1046	18%	7159	735	10%
2024	3751	486	13%	6388	835	13%
2023	3246	365	11%	6588	689	10%
2022	2960	358	12%	5989	628	10%
2021	2061	308	15%	3811	538	14%

Table 2 for Dengue and malaria Co-infected cases details for year, month, gender, Dengue Ns1/IgM results and Plasmodium species seen under peripheral smear along with available data for Dengue serotypes for the samples The patients range widely in age (from a 4-year-old to 60-year-olds) and include both males (M) and females (F). The vast majority of cases occurred in August, which aligns with peak monsoon or post-monsoon seasons when mosquito vectors for both diseases are most active. While all Dengue serotypes can be serious, DEN-2 and DEN-3 are often linked to more severe complications like Dengue Hemorrhagic Fever.

Table 2: Dengue and malaria Co-infected cases details along with available data for Dengue serotypes for the samples

Year	Month	Age	Sex	Dengue test positive by IgM capture ELISA	Dengue test positive by NS1 antigen ELISA	Malaria testing results by peripheral blood smear	Dengue serotypes results
2021	August	21	M	Positive	NA	*P. vivax* ring (2+)	NA*
2022	August	30	M	Positive	NA	*P. falciparum* gametocytes	NA*
2022	August	37	M	NA	Positive	*P. vivax* ring (2+)	NA*
2023	August	60	F	NA	Positive	*P. vivax* (3+)	DEN-3

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2024	March	30	M	Positive	NA	*P. falciparum* ring (3+)	NA*
2024	July	28	M	Positive	NA	*P. falciparum* ring (3+)+gametocyte	NA*
2024	August	50	M	Positive	NA	*P. vivax* ring +trophozoites	NA*
2024	September	30	M	NA	Positive	*P. falciparum* ring (3+)+gametocyte	DEN-1
2024	August	16	F	NA	Positive	*P. vivax* ring (3+) + trophozoites	DEN-3
2024	August	25	F	NA	Positive	*P. vivax* ring (1+) + trophozoites	DEN-2
2024	August	18	F	Positive	NA	*P. vivax* ring (1+) + trophozoites	DEN-2
2024	August	50	M	Positive	NA	*P. falciparum* ring (2+)+gametocyte	NA*
2024	August	20	M	Positive	NA	*P. vivax* ring (2+) + trophozoites	DEN-1
2024	August	4	F	NA	Positive	*P. falciparum* ring (2+)	NA*
2024	August	60	F	NA	Positive	*P. falciparum* ring (2+)	NA*
2024	August	24	M	NA	Positive	*P. vivax* ring (1+) + trophozoites	DEN-3
2024	August	22	F	Positive	NA	*P. vivax* ring +trophozoites	DEN-2
2025	July	30	F	NA	Positive	*P. falciparum* ring (3+)	NA*
2025	June	NA	NA	NA	Positive	*P. falciparum* ring (3+)	NA*
2025	May	NA	NA	Positive	NA	*P. vivax* (3+)	NA*

**NA: Not applicable*

DISCUSSION

Data shows a heavy concentration of cases in August, which is consistent with studies from India and Pakistan that report a surge in co-infections between July and October/November. This "peak season" is driven by environmental factors like high rainfall and humidity, which create ideal breeding grounds for both mosquito vectors. Our data shows patients aged 4 to 60, published articles emphasize that co-infections are especially prevalent among young people and males. Some studies suggest older patients may have lower co-infection rates, possibly due to accumulated immunity [9,10]. The "mixed" nature of both Dengue and Malaria is a documented phenomenon in endemic regions. Reported prevalence rates vary widely, from as low as 1.5–3% in some Indian studies to as high as 22–25% in regional tertiary care centers [8-12]. Present study identifies both *P. vivax* and *P. falciparum*. Literature notes that *P. vivax* is often the predominant malaria species in these co-infections, though *P. falciparum* is frequently associated with more severe clinical outcomes. The presence of DEN-1, DEN-2, and DEN-3 in present data matches reports of multiple circulating serotypes in urban areas, which can lead to more complex immune responses. A clear seasonal pattern emerges from the data, as the overwhelming majority of cases are recorded in August. This suggests a peak in mosquito-borne disease transmission during or immediately following the monsoon season. The affected population is diverse, spanning from young children (e.g.,

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a 4-year-old female) to older adults (e.g., 60-year-olds), with no specific age or gender appearing to be exempt. Overall a significant public health challenge involving the simultaneous occurrence of two major mosquito-borne illnesses in the same individuals during peak transmission month [14,15].

Dengue–malaria co-infection is an emerging clinical and epidemiological concern in tropical and subtropical regions where both Aedes and Anopheles vectors coexist. Although the prevalence of co-infection in India and other endemic countries is relatively low, ranging between 2–5% in most hospital-based series, reports suggest that the true burden may be underestimated due to diagnostic challenges and overlapping clinical features [6–12]. The concurrent presence of both pathogens complicates clinical management, as shared symptoms such as fever, thrombocytopenia, hepatomegaly, and altered liver function tests often make differentiation difficult [6–8].

The role of dengue serotypes adds another layer of complexity in co-infection. Circulating serotypes vary across regions and time, with DENV-2 and DENV-3 often implicated in more severe clinical presentations such as dengue hemorrhagic fever and dengue shock syndrome [14]. In the context of malaria co-infection, where systemic inflammation and hematological abnormalities are already present, infection with virulent dengue serotypes may worsen clinical outcomes. For example, Plasmodium falciparum malaria itself is associated with severe anemia, hepatic dysfunction, and cerebral involvement; when coupled with a severe dengue serotype, the risk of complications such as bleeding, multi-organ failure, and prolonged hospitalization increases [4,5,9,10].

Another consideration is the immunological interaction between malaria parasites and dengue serotypes. Secondary dengue infections with heterologous serotypes are associated with antibody-dependent enhancement (ADE), which leads to higher viral loads and more severe disease [13]. Malaria-induced immune activation may further amplify this effect, creating a synergistic pathophysiological response. This highlights the need to consider both the infecting Plasmodium species and the circulating dengue serotype when interpreting disease severity in co-infected patients [5,14].

From a public health perspective, serotype surveillance in co-endemic areas is essential to anticipate potential surges of severe co-infection cases. Integrated vector management strategies should address both Aedes and Anopheles mosquitoes, as seasonal overlaps in transmission contribute significantly to co-infection risk [12,15]. Furthermore, routine diagnostic protocols in febrile illness clinics should include both malaria and dengue testing, particularly during peak transmission seasons, to avoid misdiagnosis and ensure appropriate treatment.

LIMITATIONS

- Small sample size
- Retrospective design
- Dengue serotyping available only for a subset of cases
- Only dengue-positive cases were traced for malaria, introducing selection bias
- Lack of clinical severity and outcome data

CONCLUSIONS

In conclusion, dengue–malaria co-infection, though uncommon, poses significant diagnostic and therapeutic challenges. The severity and outcomes of such cases may be influenced by the circulating dengue serotype, especially DENV-2 and DENV-3, in conjunction with the Plasmodium species involved. Strengthening laboratory diagnostics, enhancing serotype surveillance, and implementing integrated vector control are critical steps to mitigate the public health impact of this dual infection.

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