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Original Research Article

Seroprevalence and co-infection of dengue and chikungunya viruses: a cross-sectional study from Southern Assam

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ABSTRACT

Background: Dengue virus (DENV) and chikungunya virus (CHIKV) transmitted by *Aedes aegypti* and *Aedes albopictus*, are responsible for overlapping febrile illnesses in tropical regions such as India. In Assam, these infections remain underreported despite their clinical severity and epidemiological significance. The study aimed to determine the seroprevalence of DENV and CHIKV, including co-infections, among patients with prolonged febrile illness and to analyze their demographic and clinical characteristics in a tertiary care hospital of South Assam.
Methods: A hospital-based cross-sectional study was conducted at Viral Research and Diagnostic Laboratory (VRDL), Silchar Medical College and Hospital, Assam, from January to December 2024. A total of 250 patients presenting with >5 days of high-grade fever (>39°C) accompanied by chills, rashes, arthralgia, or myalgia were enrolled. Serum samples were tested using IgM capture enzyme-linked immunosorbent assay (ELISA) kit, for dengue and chikungunya. Ethical approval and informed consent were obtained. Statistical analysis was performed using Microsoft Excel ($p<0.05$).
Results: The prevalence of DENV was 17.2% (43/250), CHIKV 19.2% (48/250), and co-infection 3.6% (9/250). Clinical manifestations among co-infected cases included fever (100%), headache (44.4%), arthralgia (33.3%), and myalgia (33.3%). Thrombocytopenia was observed in 62.9% of dengue positive patients. Demographic associations were statistically non-significant, though descriptive trends indicated peak incidence during June-July, male predominance (77.8%), and highest burden among individuals aged 21-30 years (55.6%).
Conclusions: The study highlights a considerable burden of DENV and CHIKV infections, with notable co-infection rates in northeastern India. Concurrent testing, strengthened surveillance, vector control, and public health awareness are essential to mitigate the dual threat posed by these arboviruses.

Keywords: Dengue, Chikungunya, Coinfection, Seroprevalence, Arboviruses

INTRODUCTION

Arboviral infections remain a significant public health burden in tropical and subtropical regions worldwide. Dengue virus (DENV) and chikungunya virus (CHIKV), transmitted primarily by *Aedes* mosquitoes, are responsible for millions of infections annually and cause acute febrile illnesses with overlapping clinical manifestations.[1-3] DENV, a member of the family Flaviviridae and genus *Flavivirus*, exists as five serotypes (DENV-1 to DENV-5) and possesses a positive-sense, single-stranded RNA genome of approximately 11 kb encoding three structural proteins (C, prM, and E) and seven non-structural proteins (NS1, NS2B, NS3, NS4A, NS4B, and NS5).[5,6] CHIKV, classified under the family Togaviridae and genus *Alphavirus*, comprises three genetic lineages (Asian, East/Central/South African, and West African) with a similar genome organization encoding three structural proteins (C, E1, E2) and four non-structural proteins (nsP1-nsP4).[4]

Clinically, both dengue and chikungunya present with acute-onset high-grade fever, headache, myalgia, arthralgia, maculopapular rash, and gastrointestinal symptoms, making clinical differentiation challenging.[2] However, management strategies differ significantly; non-steroidal anti-inflammatory drugs are widely used for symptomatic relief in chikungunya but are contraindicated in dengue due to the risk of bleeding complications and progression to dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS).[6,7] Laboratory confirmation is therefore essential for accurate diagnosis and appropriate clinical management. Available diagnostic methods include rapid immunochromatographic tests, serological assays (MAC-ELISA for IgM antibody detection and hemagglutination inhibition tests), antigen detection (NS1), and nucleic acid amplification techniques such as reverse transcription polymerase chain reaction (RT-PCR), each with specific windows of detection and inherent limitations.

In India, both dengue and chikungunya are endemic in numerous states, including Assam, where dengue is regularly reported through surveillance systems while chikungunya often remains underdiagnosed due to limited laboratory facilities and insufficient clinical awareness. Although multiple studies from other Indian regions have documented DENV-CHIKV co-infections with varying prevalence rates, comprehensive data from Southern Assam remain scarce.[8,9] Given the shared vector ecology, overlapping geographical distribution, and concurrent circulation of both viruses, the occurrence of simultaneous infections in the same host is epidemiologically plausible and may potentially alter disease severity, clinical course, and prognostic outcomes.

The aim of this study was to determine the seroprevalence of dengue and chikungunya infections, including their co-infection, among febrile patients attending a tertiary care centre in Southern Assam, and to analyze associated demographic and clinical characteristics in order to generate baseline epidemiological data.

METHODS

Study design and setting

A hospital-based, cross-sectional observational study was conducted in the Viral Research and Diagnostic Laboratory (VRDL), Department of Microbiology, Silchar Medical College and Hospital (SMCH), Silchar, Assam, over a 12-month period from January to December 2024. SMCH is a tertiary care referral centre serving Southern Assam and adjoining regions, making it an appropriate setting for investigating arboviral infections in this endemic zone.

Study population and sample size calculation

Eligible participants included patients aged 5-80 years attending the Medicine and Paediatrics outpatient departments or admitted to the corresponding inpatient wards with clinical suspicion of dengue or chikungunya based on presenting symptoms.

Inclusion criteria

Patients presenting with fever >39°C persisting for more than five days, accompanied by at least one of the following manifestations-rash, headache, arthralgia, myalgia, nausea, or vomiting.

Exclusion criteria

Patients or their attendants who refused to provide informed consent and those who are already diagnosed positive in the laboratory for other causes of fever.

Sample size

Sample size was calculated based on an expected dengue prevalence of 19%, allowable error of 5%, and 95% confidence interval, yielding a minimum of 236 subjects; 250 consecutive eligible patients were enrolled to account for potential dropouts or invalid samples.[10]

Ethical approval and informed consent

The study protocol was submitted to and approved by the Institutional ethics committee of Silchar Medical College and Hospital prior to patient enrolment. Written informed consent was obtained from all participants.

Specimen collection and processing

Approximately 5 ml of venous blood was collected aseptically from each participant into sterile plain vacutainers (clot activator tubes). Samples were allowed to clot for 30 minutes at room temperature, then centrifuged at 3000 rpm for 5 minutes to separate serum. Serum aliquots were transferred into labelled sterile microcentrifuge tubes and stored at -20°C until serological testing. Platelet counts and other parameters were obtained from the hospital's central clinical laboratory as part of routine diagnostic workup.

Serological testing methodology

All the samples were tested for Anti-DENV and anti-CHIKV IgM antibodies using MAC-ELISA kits provided by the National Institute of Virology, Pune, following the manufacturer's instructions. Optical density (OD) was measured spectrophotometrically at 450 nm using a microplate reader. Test results were interpreted using the following OD cut-offs: negative if OD <2× negative control (NC); positive if OD >3× NC; and equivocal if OD fell between these thresholds. Equivocal results were retested in duplicate. Internal quality control requirements specified that negative control OD should be <0.18 and positive control OD should be ≥6 times the negative control OD for valid test runs.

Data collection and statistical analysis

Clinical and demographic data including age, sex, residence (urban/rural/peri-urban), detailed symptom profile (fever, rash, headache, arthralgia, myalgia, nausea, vomiting), duration of illness, travel history and relevant laboratory parameters (platelet count) were systematically recorded on a structured proforma. Data were entered into Microsoft Excel spreadsheet and analysed using statistical package for the social sciences (SPSS) version 22.0. Categorical variables were compared between groups using the Chi-square test (or Fisher's exact test where cell counts were <5), and two-tailed p values <0.05 were considered statistically significant. Descriptive statistics including frequencies, percentages, and proportions were calculated for seroprevalence estimates.

RESULTS

Of the 250 patients enrolled**,** dengue monoinfection was detected in 34 (13.6%), chikungunya monoinfection in 39 (15.6%), and DENV-CHIKV co-infection in 9 (3.6%). Accordingly, the overall seroprevalence of dengue (including co-infected cases) was 17.2% (43/250), while that of chikungunya was 19.2% (48/250) (Table 1).

Table 1: Seroprevalence of dengue, chikungunya, and their co-infection.

Infection category	Number of patients (N)	Prevalence (%)
Dengue monoinfection	34	13.6
Chikungunya monoinfection	39	15.6
DENV-CHIKV co-infection	9	3.6

Table 2: Demographic characteristics of Co-infected cases (n=9).

Demographic parameter	Number (%)	P value
Age 21-30 years	5 (55.6)	0.36
Age >30 years	4 (44.4)	
Males	7 (77.8)	0.34
Females	2 (22.2)	

Clinical presentation in co-infected cases

Among the nine patients with DENV-CHIKV co-infection, clinical manifestations were documented as follows: fever was present in all co-infected patients (9/9; 100%), headache in 4 patients (44.4%), arthralgia in 3 patients (33.3%), myalgia in 3 patients (33.3%), and rash in 2 patients (22.2%) (Table 2). Chi-square analysis revealed a statistically significant association between co-infection status and the presence of fever with headache (p=0.042) compared to monoinfected patients. Other symptoms such as nausea and vomiting were less frequently reported and did not show significant differences between co-infected and monoinfected groups.

Regarding haematological parameters, thrombocytopenia (defined as platelet count <1.5 lakh/mm³ or <150 × 10^9/l) was observed in 27 of 43 dengue-positive patients (62.9%), including both dengue monoinfected and coinfected individuals. The association between dengue seropositivity and thrombocytopenia was highly statistically significant (p<0.0001).

Temporal and demographic distribution

The temporal distribution of cases showed clustering during the monsoon season, with cases reported throughout the 12-month study period. For coinfected patients specifically, one-third (3/9; 33.3%) presented during June, followed by 2 cases (22.2%) in July. This seasonal pattern corresponds with the onset and progression of monsoon rains in the region, which favour increased breeding of *Aedes* mosquitoes in water collections around human habitations.

Demographic analysis of the nine co-infected patients revealed that 7 cases (77.8%) were males and 2 were females, and 5 cases (55.6%) fell within the 21-30-year age group, with the remaining distributed across other age strata. Chi-square analysis demonstrated that neither age (p=0.36) nor sex (p=0.34) showed a statistically significant association with coinfection status, though the small number of coinfected cases limits the statistical power of these comparisons. Most patients were residents of urban or peri-urban localities known to have dense *Aedes* populations and breeding habitats, though detailed entomological and environmental data were not systematically collected.

DISCUSSION

This study provides important baseline epidemiological data on the seroprevalence of dengue and chikungunya infections and their coinfection among febrile patients in Southern Assam. The dengue prevalence of 17.2% and chikungunya prevalence of 19.2% are broadly consistent with reports from other endemic regions of India, where published seroprevalence rates typically range from 12% to 20% in hospital-based studies.[11,12] The slightly elevated chikungunya prevalence observed in this study (19.2%) relative to some previous regional reports (which range from 5% to 8.5%) suggests increasing epidemiological activity of CHIKV in Assam, potentially reflecting expanded vector distribution or increased viral circulation in recent years.[13,14] This finding corroborates clinical observations and unpublished surveillance data indicating a rising trend of chikungunya cases in Northeast India.

The coinfection rate of 3.6% in this study aligns with several published studies reporting coinfection frequencies of 2.7% to 4.5%, but remains notably lower than reports from some Northeast Indian outbreaks, which have

documented coinfection rates approaching 19-26%.[13,15] This discrepancy may be attributable to differences in study design (hospital-based versus community-based), diagnostic methods (serology versus molecular), timing of case ascertainment relative to illness onset, and local epidemiological circumstances. The IgM-based approach used in the present study is most sensitive during the acute phase and early convalescent period (typically day 4-30 of illness), which may underestimate true coinfection rates if patients present very early or late in the illness course.

The near-universal presence of fever (100%) in coinfected patients is consistent with previous studies describing fever as the cardinal feature of both dengue and chikungunya infections.[10,16] The significant association of fever with headache in coinfected cases ($p=0.042$), while statistically significant, has limited discriminatory value given the non-specific nature of these symptoms in acute viral febrile illnesses. The relatively lower frequency of rash (22.2%) in coinfected cases is noteworthy; rash is typically prominent in chikungunya but less common in some dengue infections, and the reduced proportion here may reflect the complex interplay of immune responses when both viruses are simultaneously present.

The striking finding of thrombocytopenia in 62.9% of dengue-positive patients ($p<0.0001$) is well established in the literature and reflects the multifactorial pathophysiology of dengue, including bone marrow suppression, immune-mediated platelet destruction via antigen-antibody complexes, and increased peripheral platelet consumption.[13] This association underscores the clinical significance of platelet monitoring in dengue cases and the risk of haemorrhagic complications in severe disease. Although the present study did not specifically investigate whether coinfection exacerbates thrombocytopenia compared to dengue monoinfection alone, the high proportion of thrombocytopenia among dengue-positive cases warrants close clinical monitoring in coinfected patients.

The male predominance (77.8%) and concentration of coinfections in the 21-30 year age group (55.6%) is consistent with prior reports, which attribute such demographic patterns to greater outdoor exposure, occupational activities in rural or semi-rural settings, and higher vector contact rates among young adult males.[11,13,17] However, the lack of statistical significance for these demographic factors in relation to coinfection suggests that while the apparent clustering may reflect healthcare-seeking behaviour and enrolment bias, the true risk of coinfection is likely driven primarily by vector exposure intensity and environmental factors rather than age or sex per se.

The pronounced seasonality, with peak coinfections occurring in June and July, corresponds precisely with the onset of monsoon rainfall in Assam and the known ecology of *Aedes* mosquitoes. These mosquitoes thrive in clean, stagnant water collections (flower pots, discarded containers, coconut husks, roof gutters) that accumulate during rainy seasons, leading to explosive population growth and increased human-vector contact. This seasonal pattern reinforces current vector control strategies, particularly the importance of pre-monsoon and monsoon-period interventions including source reduction, larval surveillance, and community mobilisation.

Limitations and strengths

Several limitations warrant discussion. First, this was a hospital-based study conducted at a single tertiary centre, which may limit the generalisability of findings to the broader community setting where many infections likely remain undiagnosed or unreported. Second, the reliance on serology alone (IgM detection) without confirmatory molecular testing (RT-PCR) cannot definitively exclude past infections reactivating or late presentations with residual IgM, though the >5-day fever criterion enriches for acute infections. Third, the absence of systematic severity grading using WHO dengue classifications (DHF/DSS) precludes firm epidemiological conclusions regarding whether coinfection increases risk of severe disease compared to monoinfection. Fourth, small sample size (n=9 coinfected) limits statistical power for meaningful subgroup analyses and associations.

The study's strengths include being the first systematic investigation of DENV-CHIKV coinfection from Southern Assam, a previously understudied region, thereby addressing a knowledge gap. The use of standardised NIV Pune ELISA kits ensures comparability with other Indian laboratory studies. Systematic data collection on demographics, symptoms, and laboratory parameters provides a comprehensive epidemiological profile. The study highlights the practical need for simultaneous testing of both viruses in endemic settings to guide evidence-based case management.

CONCLUSION

This hospital-based serological study reveals that dengue and chikungunya viruses are co-endemic in Southern Assam, with significant burdens of both monoinfections and measurable coinfections among febrile patients. The seroprevalence of dengue (17.2%) and chikungunya (19.2%), along with a coinfection rate of 3.6%, reflects substantial viral circulation in this region. Although the overall coinfection rate remains relatively low, the rising prevalence of chikungunya (19.2%) signals an emerging public health challenge requiring increased clinical vigilance and strengthened laboratory diagnostic capacity. Coinfected patients most commonly presented with non-specific febrile illness during the monsoon season (June-July), predominantly affecting young adult males, with a high proportion demonstrating dengue-associated thrombocytopenia.

Given the overlapping clinical presentations and divergent management implications-particularly the

contraindication of NSAIDs in dengue due to bleeding risk and the importance of monitoring for haemorrhagic progression-simultaneous serological testing for dengue and chikungunya should be implemented as a standard protocol in dengue-endemic hospitals within Assam. Clinicians must maintain a high index of suspicion for coinfection, especially during monsoon months when vector populations peak. Future research employing molecular methods (RT-PCR for serotyping/genotyping), prospective long-term follow-up of coinfected patients, and community-based serosurveys would provide valuable insights into the true burden and clinical consequences of coinfection. Strengthening laboratory diagnostics, enhancing clinician awareness through continuing medical education, implementing rigorous vector control measures targeting *Aedes* breeding sites, and promoting community participation in environmental management remain essential pillars of arboviral disease prevention and control in this endemic region.

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