

PREVALENCE AND CLINICAL PROFILE OF DENGUE SEROTYPE CO-INFECTION AMONG SUSPECTED DENGUE PATIENTS

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ABSTRACT

Dengue fever is an important arboviral illness caused by dengue virus (DENV), which has four antigenically distinct serotypes, DENV-1 to DENV-4. Northern India continues to experience repeated dengue outbreaks, with a steadily rising disease burden. In Western Uttar Pradesh, the circulation of other arboviruses, especially chikungunya virus (CHIKV), has not been adequately investigated because dengue and chikungunya produce similar clinical features and are transmitted by the same mosquito vectors. Information on circulating dengue serotypes and arboviral co-infections from this region is still limited.

This hospital-based cross-sectional study was conducted at a tertiary care centre in North India to assess the serological and molecular epidemiology of dengue infection, identify circulating dengue serotypes, and estimate the burden of chikungunya infection among patients presenting with dengue-like illness. Clinically suspected dengue cases were screened for dengue NS1 antigen and anti-dengue IgM antibodies by ELISA. Seropositive samples were further analyzed by multiplex RT-PCR for molecular detection and serotyping. Anti-chikungunya IgM testing was also performed to evaluate dengue–chikungunya co-infection.

Among 375 suspected cases, 96 (25.6%) were serologically positive for dengue. Of 50 samples tested for CHIKV, 10 (20.0%) were positive, including 3 (6.0%) concurrent dengue–chikungunya infections. Dengue RNA was detected and serotyped in 60 cases. DENV-3 was most common (41.7%), followed closely by DENV-2 (40.0%), while DENV-1 accounted for 3.3%. No DENV-4 was detected. Mixed-serotype infection occurred in 15.0% of RT-PCR-positive samples. These findings show active co-circulation of multiple dengue serotypes and emphasize the need for integrated surveillance.

KEYWORDS: Dengue virus; DENV-1-4, Serotype, RT-PCR, Co-infection, Arboviruses; Molecular characterization

INTRODUCTION

Arboviruses (arthropod-borne viruses) are important pathogens responsible for millions of infections in humans and animals worldwide.¹ Among the most clinically significant arboviruses are dengue virus (DENV), chikungunya virus (CHIKV), Zika virus (ZIKV), and Japanese encephalitis virus (JEV), all of which co-circulate in tropical and subtropical regions. These viruses cause illnesses with overlapping clinical manifestations, particularly during the early stages of infection, making differential diagnosis challenging in endemic areas.

Uttar Pradesh state (UP) is marked as the most populated state of India with high dengue transmission. Dengue is most prevalent and epidemiologically important arboviral infection worldwide.^{2-3,6,8} India experiences a considerable dengue burden, accounting for an estimated 12.9 million primary infections annually. The four DENV serotypes (1-4) are antigenically distinct but epidemiologically similar, while genetically divergent. Primary infection with one DENV serotype confers lifelong immunity against that specific serotype (homologous protection), but offers only short-term, partial immunity against the other serotypes (heterologous protection), potentially predisposing individuals to more severe disease upon secondary infection.^{4,5} Moreover, concurrent infections with multiple DENV serotypes have been increasingly reported and are considered epidemiologically important because they may be associated with an increased risk of severe dengue.^{9,10} The simultaneous circulation of multiple dengue virus serotypes and their associated immunological interactions complicate both disease management and vaccine development.¹¹

Chikungunya fever, caused by the Chikungunya virus (CHIKV), remained underreported or epidemiologically silent in recent years due to insufficient surveillance and limited diagnostic usage. No data/limited data is available about chikungunya prevalence in the region. The emergence and re-emergence of these arboviral diseases are driven by a variety of complex factors, including viral recombination and mutation, which lead to the development of more virulent and adaptable strains; urbanization and human activities that create conducive environments for vector-host interactions; and increased air travel and global commerce, which facilitate the geographic spread of these viruses. Knowing co-circulation patterns is vital, as the high frequency of co-

infections and distinct age-specific incidence patterns necessitate expanding molecular diagnostic capacity within surveillance networks¹⁶ Understanding these complex interactions is essential, as the clinical presentation of coinfections can mimic single-pathogen illness, frequently resulting in misdiagnosis within endemic regions.¹⁷ The 1963 hemorrhagic fever epidemic in Calcutta was the first of its kind, and was attributed to the dual circulation of Dengue and Chikungunya viruses.^{4,5} Studies suggest that co-infections of Dengue and Chikungunya viruses can be associated with greater clinical severity compared to monoinfections.^{15,17-18} The increasing use of data-driven and machine-learning approaches has created opportunities for improving disease detection, prediction, and evidence-based clinical decision-making¹⁹. Consequently, prioritizing the implementation of robust, high-throughput molecular surveillance remains critical for distinguishing between these viruses and identifying potential changes in pathogen virulence.²⁰ The deployment of multiplex assays, such as any arboviral panel offers a scalable solution to improve diagnostic accuracy and reduce the prevalence of undetected co-infections as demonstrated in a similar study.²¹ The present study aimed to investigate the serological and molecular epidemiology of dengue infection among patients presenting with acute febrile illness in Western Uttar Pradesh, India. The study also assessed the burden of chikungunya virus (CHIKV) infection and the occurrence of dengue-chikungunya co-infection. Additionally, the study aimed to improve understanding of the circulation types of dengue virus in Western Uttar Pradesh, which has been responsible for several outbreaks in this region in the past.

MATERIALS AND METHODS

Study Design and Setting

A hospital-based cross-sectional study was conducted at the virology laboratory of a tertiary care center of western Uttar Pradesh, India. Patients presenting with acute febrile illness and clinically suspected for dengue infection (high-grade fever ($>39^{\circ}\text{C}$), chills, rash, arthralgia, joint swelling, headache, myalgia, retro-orbital pain, nausea, and vomiting) were included in the study following referral from treating clinicians. Demographic and clinical details were recorded using a structured case record form.

Approximately 5–10 mL of venous blood was collected aseptically from each patient. Samples were transported to the laboratory within 24 hours under cold-chain conditions maintained at $2\text{--}8^{\circ}\text{C}$. Samples intended for molecular analysis were preserved at -80°C after RNA extraction.

Serological Diagnosis

Serological testing for dengue infection was performed using enzyme-linked immunosorbent assay (ELISA) based methods. Patients presenting within the first five days of fever onset were tested for dengue NS1 antigen using the NS1 Ag MICROLISA kit manufactured by J. Mitra & Co. Pvt. Ltd and the samples collected after five days of illness were screened for anti-dengue IgM antibodies using IgM antibody capture ELISA kits developed by National Institute of Virology. All samples were additionally tested for anti-chikungunya IgM antibodies using NIV IgM capture ELISA kits. All assays were performed according to the manufacturer's instructions, and appropriate positive and negative controls were included in each run for quality assurance. The reported sensitivity and specificity for the NIV dengue IgM capture ELISA kit was 98.53% and 98.84%, for NS1 Ag MICROLISA was 99.69% and 100% while for chikungunya IgM capture ELISA was 95.0% and 98.0%, respectively.

Dengue Serotyping by RTPCR

All ELISA-confirmed dengue-positive samples (NS1 antigen and/or IgM antibody positive) were subjected to viral RNA extraction using a commercial viral RNA extraction kit according to the manufacturer's protocol. Extracted RNA was used immediately or stored at -80°C until further molecular processing.

Dengue virus serotyping was performed using the DiAGSure Dengue Genotyping Kit v2.0 manufactured by GCC Biotech (India) Pvt. Ltd. The assay is a TaqMan-based real-time reverse transcription polymerase chain reaction (qRT-PCR) designed for qualitative detection and differentiation of DENV serotypes 1–4. Multiplex qRT-PCR was carried out as per the manufacturer's instructions targeting the dengue virus polyprotein (POLY) gene for each genotype. An endogenous RNase-P internal control was included in each reaction to assess sample quality and extraction efficiency.

Ethical Considerations

Ethical Approval was obtained from the Institutional Ethics Committee prior to commencement of the study (Letter no. SNMC/IEC/2024/317). Patient confidentiality was maintained throughout the study, and all data were anonymized prior to analysis.

RESULTS

A total of 375 serum samples from patients presenting with dengue-like illness were enrolled. Based on the duration of illness, 325 (86.7%) samples from patients presenting within ≤ 5 days of fever were screened for dengue NS1 antigen, whereas 50 (13.3%) samples from patients presenting after 5 days of fever were tested for anti-dengue IgM antibodies using ELISA. Of the 375 samples tested, 96 (25.6%) were positive for dengue infection. Among the dengue-positive samples, 87/96 (90.6%) were positive for dengue NS1 antigen, while 9/96 (9.4%) were positive for anti-dengue IgM antibodies. (Table 1)

Table 2. Serological and molecular findings among study participants (n = 375)

Parameter	Number (n)	Percentage (%)
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Total samples tested	375	100.0
Dengue-positive	96	25.6
NS1 positive	87	23.2
Dengue IgM positive	9	2.4
RT-PCR positive	60	16.0
CHIKV positive*	10	20.0
DENV-CHIKV co-infection*	3	6.0

* Among 50 samples tested for CHIKV IgM.

Of the 96 dengue-positive patients, males constituted 64.6% (62/96) and females 35.4% (34/96), yielding a male-to-female ratio of 1.8:1. The age of the patients ranged from 4 to 62 years. The majority of patients belonged to the ≥ 18 years' age group (68.8%, 66/96), followed by the 8–17 years (19.8%, 19/96) and 0–7 years (11.5%, 11/96) age groups. (Table 2)

Table 2. Demographic characteristics of dengue-positive patients (n = 96)

	Category	Number (n)	Percentage (%)
Gender	Male	62	64.6
	Female	34	35.4
Age group (years)	0–7	11	11.5
	8–17	19	19.8
	≥ 18	66	68.8
Total		96	100.0

Dengue viral RNA was successfully detected and serotyped in 60 of the 96 serologically positive samples (62.5%). Molecular characterization demonstrated the co-circulation of DENV-3 and DENV-2, with DENV-3 being the most frequently detected serotype, identified in 25/60 (41.7%) cases, closely followed by DENV-2 in 24/60 (40.0%) cases. DENV-1 was detected in 2/60 (3.3%) cases, while no DENV-4 infection was identified during the study period. Mixed serotype infections were observed in 9/60 (15.0%) RT-PCR-positive samples, comprising DENV-1/DENV-3 co-infection in 4/60 (6.7%) cases and DENV-2/DENV-3 co-infection in 5/60 (8.3%) cases.

Screening for anti-chikungunya IgM antibodies was performed on 50 samples collected from patients presenting after 5 days of fever. CHIKV infection was detected in 10 (20.0%) samples, including 7 (14.0%) cases of CHIKV mono-infection and 3 (6.0%) cases of concurrent dengue–chikungunya infection. Serotype analysis revealed that all three co-infected patients were infected with DENV-3.

DISCUSSION

Dengue continues to pose a major public health challenge in tropical and subtropical countries, particularly in India, where recurrent outbreaks and co-circulation of multiple arboviruses complicate disease surveillance and management. **Western Uttar Pradesh has emerged as an important dengue-endemic region of North India, experiencing recurrent outbreaks and sustained transmission over the past decade. Favorable climatic conditions, rapid urbanization, population movement, and the widespread distribution of Aedes vectors contribute to persistent dengue circulation in this region.**

In the present study, dengue positivity was observed in 25.6% of the clinically suspected cases. Similar positivity rates have been reported in another study from the western Uttar Pradesh region.²² The predominance of NS1 antigen positivity (90.62%) compared to dengue IgM positivity (9.3%) suggests that the majority of patients presented during the early acute phase of infection, when viral antigenemia is more readily detectable than the antibody response. This finding highlights the importance of NS1 antigen detection as an effective early diagnostic tool for dengue infection as depicted in previous studies.^{23–25} Among dengue positive cases, the majority of patients were adults aged ≥ 18 years, and males constituted the predominant affected group. The male predominance observed in the present study is in agreement with findings from other global as well as Indian studies.^{26–29} Some studies similarly reported a higher burden of dengue infection among males, with male-to-female ratios ranging from 1.9:1 to 2.5:1.^{30–32} Studies suggest that higher dengue prevalence among males may be associated with increased outdoor exposure and occupational activities, leading to greater contact with mosquito vectors.^{33–35} Similarly, the predominance of adult cases observed in the present study is consistent with several Indian studies reporting higher dengue incidence among the economically productive age group. Such trends align with recent literature identifying age-related disparities and male predominance as key indicators of transmission patterns in endemic settings.^{36–37}

Furthermore, the screening for IgM chikungunya antibodies among dengue IgM-tested samples (50/96) revealed the presence of hidden chikungunya burden (20%) including DENV/CHIKV co-infection (3.12%) aligning with the previously reported rates, which range from 2.8% to over 10%^{38–41,19} The detection of co-infections is clinically significant because dengue and chikungunya share similar clinical manifestations, including fever, rash, myalgia, and arthralgia, which may lead to diagnostic challenges and underreporting. This necessitates improved diagnostic protocols that simultaneously screen for both pathogens, particularly in resource-limited clinical settings where symptomatic overlap often obscures precise identification.⁴² Therefore, simultaneous screening for multiple arboviral infections may improve patient management and strengthen outbreak surveillance.⁴⁴

Our study showed a slight dominance of DENV-3 (41.7%) followed by DENV-2 (40%). DENV-1 was reported in 3.3% of the cases. A research conducted across multiple regions in India reported that DENV-3 is a common serotype in Western and Northern India.⁴⁵ Previously, a research within the eastern-UP region demonstrated that the 2015–2016 dengue outbreak was driven entirely by the cosmopolitan genotype of DENV-2.⁴⁶ Subsequent analysis later confirmed the localized predominance of the DENV-2 serotype, suggesting that this genotype has remained largely stable with minimal evolutionary progression.⁴⁴ DENV-4 was not detected in our study similar to other Indian studies.^{45,47,50-52}

Moreover, circulation of multiple dengue serotypes within the same geographic area is epidemiologically important, as secondary infection with a heterologous serotype has been associated with increased risk of severe dengue manifestations due to antibody-dependent enhancement. Temporal fluctuations in the predominance of dengue virus serotypes have been reported previously, with shifts in circulating serotypes occurring irregularly over time. Serotyping revealed the co-circulation of DENV-3 and DENV-2 (41.7% & 40% respectively), with only a marginal predominance of DENV-3. A comparable serotype distribution has been reported from previous studies in the region, documenting DENV-3 and DENV-2 as the major circulating serotypes, followed by DENV-1 similar to this study.⁴⁷ Similarly, Mishra et al. (2015) reported the co-circulation of three dengue virus serotypes (DENV-1, DENV-2, and DENV-3) in the Central Uttar Pradesh region during 2009–2012, highlighting the sustained circulation of multiple serotypes in northern India.⁴⁸

The occurrence of mixed-serotype dengue infections has attracted considerable attention because concurrent infection with multiple DENV serotypes may influence the clinical manifestations and severity of disease. Previous studies have suggested that simultaneous circulation and infection with different serotypes and genotypes may facilitate viral recombination, potentially contributing to the emergence of strains with altered pathogenicity or virulence.⁴⁹ In addition, dual-serotype infections in humans may result from mosquitoes harboring more than one DENV serotype, which can occur following multiple blood meals during a single gonotrophic cycle.^{50,51} High vector density and the co-circulation of multiple serotypes in endemic regions are also considered important factors promoting mixed-serotype infections. Mixed-serotype infections were detected in 15.0% of RT-PCR-positive cases, a prevalence comparable to that reported in previous studies (14% - 19%).^{50,51} The predominant combinations identified were DENV-1/DENV-3 (6.7%) and DENV-2/DENV-3 (8.3%) out of the 15% co-infections reported in this study. These findings are in agreement with previous reports from dengue-endemic regions worldwide that have documented concurrent infections with multiple dengue virus serotypes,^{50,52-56} whereas other regions such as Indo-China, Brazil, Kerala and Karnataka have reported higher rates⁵⁷⁻⁶⁰. Mixed infections may occur in hyper endemic regions where multiple serotypes circulate simultaneously and may contribute to enhanced viral evolution, disease severity, and outbreak complexity. The detection of mixed infections further emphasizes the importance of molecular surveillance and continuous monitoring of dengue virus serotype dynamics.

LIMITATIONS

The present study has several limitations. First, as a single-centre, hospital-based investigation, the findings may not be fully representative of the true community burden and epidemiological patterns of arboviral infections. However, the study was conducted at a tertiary care hospital that serves as one of the largest referral centres in the Agra Division of western Uttar Pradesh, receiving patients from both urban and rural areas across multiple districts and thereby providing a broad representation of cases from the catchment area. Second, genotypic characterization of DENV and CHIKV was not performed, limiting insights into their genetic diversity, evolutionary dynamics, and transmission patterns. Despite these limitations, the study provides important epidemiological and molecular data on circulating dengue virus serotypes and arboviral co-infections in western North India, contributing to ongoing surveillance efforts and informing public health interventions.

CONCLUSION

In conclusion, this study demonstrated a substantial burden of dengue infection among clinically suspected cases, with DENV-3 emerging as the predominant circulating serotype, followed by DENV-2 and DENV-1. The detection of mixed-serotype infections highlights the concurrent circulation of multiple dengue virus serotypes in the study area. Evidence of dengue–chikungunya co-infection was also observed, underscoring the complexity of arboviral transmission dynamics in the region. These findings emphasize the importance of continuous serological and molecular surveillance for early diagnosis, monitoring changes in circulating serotypes, detecting co-infections, and guiding effective vector control measures and public health interventions.

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Ethical Responsibilities of Authors

All authors declare that this research article is original and has not been published elsewhere in any form or language (partially or in full).

Consent to Participate

Not applicable

Consent to Publish

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Declaration of Generative AI and AI-assisted technologies in the writing process:

During the preparation of this work, the authors had not used any AI-AI assisted technologies. We both authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Data transparency

All authors agree that all data, materials as well as software application has supported their published claims and complied with field standards.

Author contributions

Dr. Priyanka Singh, Dr. Ankur Goyal, and Dr. Vikas Kumar have contributed to the study conception and design for this research work. Material's preparation, data collection and analysis were performed by scholar, Ms. Neha K. Mani and Mr. Vinay Verma. The first draft of the manuscript was written by Neha K. Mani. Dr. Priyanka Singh, Dr. Ankur Goyal, and Dr. Vikas Kumar have thoroughly reviewed and approved the final manuscript.

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