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CROSS-REACTIVITY OR DUAL INFECTION OF CHIKUNGUNYA AND DENGUE IN AN ENDEMIC AREA OF DENGUE

Dr. Shripad Taklikar[1*], Dr. Pradnya Kale[2], Dr. Arghadip Samaddar[3], Dr. Sujata Baveja[4]

[1*]Additional professor at Department of Microbiology, Lokmanya Tilak Municipal Medical College & General Hospital, Sion, Mumbai Email id- drshripadt@gmail.com
[2]Senior Resident at Department of Microbiology, Lokmanya Tilak Municipal Medical College & General Hospital, Sion, Mumbai Email id- prad.kale07@gmail.com
[3]Senior Resident at Department of Microbiology, Lokmanya Tilak Municipal Medical College & General Hospital, Sion, Mumbai Email id – argha2387@gmail.com
[4]Professor and Head at Department of Microbiology, Lokmanya Tilak Municipal Medical College & General Hospital, Sion, Mumbai Email id – smbaveja@yahoo.com

***Corresponding Author:** Dr. Shripad Taklikar
*Professor and Head at Department of Microbiology, Lokmanya Tilak Municipal Medical College & General Hospital, Sion, Mumbai Email id- drshripadt@gmail.com

Abstract

Background: Dengue and Chikungunya are significant mosquito-borne viral infections endemic in India, transmitted primarily by Aedes aegypti and Aedes albopictus mosquitoes. Due to shared vectors and overlapping symptoms, co-infections and cross-reactivity between these viruses pose diagnostic challenges.

Objectives: To determine the prevalence of cross-reactivity or dual infection of Dengue and Chikungunya viruses in an endemic area and compare findings with previous studies.

Methods: A cross-sectional study was conducted from October to December 2017 at Lokmanya Tilak Municipal Medical College and General Hospital (LTMMC & GH), Sion, Mumbai. A total of 300 patients clinically suspected of Dengue infection were tested for Dengue NS1 antigen and IgM antibodies using ELISA. Confirmed Dengue-positive samples were further tested for Chikungunya IgM antibodies using ELISA. Data were analyzed and compared with findings from previous studies.

Results: Out of 300 suspected cases, 121 (40.33%) were confirmed positive for Dengue infection. Among these, 51 (42.15%) samples were positive for Chikungunya IgM antibodies. The highest co-positivity was observed in patients with 4-7 days of fever duration (50%). These findings are higher compared to previous studies reporting co-infection rates ranging from 6% to 22%.

Conclusion: A significant proportion of Dengue-confirmed patients also tested positive for Chikungunya IgM antibodies, indicating considerable cross-reactivity or dual infection in the studied region. This emphasizes the need for accurate differential diagnosis using advanced molecular techniques to ensure appropriate clinical management.

Keywords: *Dengue, Chikungunya, Dual infection, Cross-reactivity, ELISA, Co-infection.*

Introduction

Mosquito-borne diseases constitute a major public health concern worldwide, particularly in tropical and subtropical regions. Among these, Dengue and Chikungunya viruses are of significant importance due to their high morbidity rates and potential for outbreaks.

Dengue virus (DENV), belonging to the family *Flaviviridae* and genus *Flavivirus*, has five distinct serotypes (DEN-1 to DEN-5) [1]. Infection with one serotype provides lifelong immunity against that serotype but only partial and temporary cross-immunity to others. Subsequent infections with different serotypes increase the risk of severe dengue manifestations. Globally, an estimated 390 million Dengue infections occur annually, with 96 million presenting clinically [2]. In India, Dengue cases have been on the rise, with 157,220 cases and 250 deaths reported in 2017 alone [3].

Chikungunya virus (CHIKV), a member of the family *Togaviridae* and genus *Alphavirus*, is transmitted by the same vectors as DENV, namely *Aedes aegypti* and *Aedes albopictus*. CHIKV causes acute febrile illness characterized by severe joint pain, which can persist for months [4]. India has experienced several Chikungunya outbreaks, with 64,057 cases reported in 2016 [5].

The co-circulation of DENV and CHIKV in endemic regions leads to the possibility of co-infections and cross-reactivity between the two viruses. This overlap complicates clinical diagnosis and management due to similar clinical presentations and serological cross-reactivity [6,7]. Accurate diagnosis is crucial for effective patient management and outbreak control.

This study aims to assess the extent of cross-reactivity or dual infection of Dengue and Chikungunya viruses in an endemic area and compare the findings with previous studies to better understand the epidemiology and inform diagnostic strategies.

Materials and Methods

Study Design and Setting

A cross-sectional study was conducted over three months from October to December 2017 at Lokmanya Tilak Municipal Medical College and General Hospital (LTMMC & GH), Sion, Mumbai, a tertiary care hospital catering to a large urban population.

Study Population

A total of 300 patients presenting to the fever outpatient department (OPD) with clinical suspicion of Dengue infection were enrolled in the study. Inclusion criteria were patients of all ages and genders presenting with high-grade fever (≥38.5°C) and at least two of the following symptoms: severe headache, retro-orbital pain, myalgia, arthralgia, rash, or hemorrhagic manifestations. Patients with known chronic illnesses or other confirmed infections were excluded.

Sample Collection

Blood samples (5 mL) were collected aseptically from each patient. Serum was separated by centrifugation and stored at -20°C until further analysis.

Laboratory Testing

All samples were initially tested for Dengue NS1 antigen and Dengue IgM antibodies using ELISA kits.

- **Dengue NS1 Antigen ELISA:** Performed using RecombiLISA Dengue NS1 Antigen ELISA kit (Lot No: E0916N2D00; Expiry Date: 2019/03/17) with a diagnostic sensitivity of 100% and specificity of 97.8%.
- **Dengue IgM Antibody Capture ELISA (MAC-ELISA):** Conducted using the National Institute of Virology (NIV) Dengue IgM Capture ELISA kit (Batch No: 17-036; Expiry Date: 06/02/18) with a diagnostic sensitivity of 98.53% and specificity of 98.84%.

Samples positive for both Dengue NS1 antigen and Dengue IgM antibodies were further tested for Chikungunya IgM antibodies.

- **Chikungunya IgM Antibody Capture ELISA (MAC-ELISA):** Performed using the NIV Chikungunya IgM Capture ELISA kit (Batch No: 17-042; Expiry Date: 28/02/18) with a diagnostic sensitivity of 95% and specificity of 98%.

All tests were conducted following the manufacturer's instructions. Positive and negative controls were included in each run to ensure quality control.

Data Analysis

Data were entered into Microsoft Excel and analyzed using SPSS version 20. Descriptive statistics were used to summarize the data. The prevalence of co-infection or cross-reactivity was calculated, and comparisons were made with previous studies using relevant literature.

Ethical Considerations

The study was approved by the Institutional Ethics Committee of LTMMC & GH, Sion. Informed consent was obtained from all participants or their guardians in the case of minors.

Results

Demographic Characteristics

Out of 300 patients screened, 121 (40.33%) were confirmed to have Dengue infection based on positive results for both Dengue NS1 antigen and Dengue IgM antibodies. Among these, 80 (66.11%) were males, and 41 (33.88%) were females, with a male-to-female ratio of approximately 2:1. The age of patients ranged from 5 to 65 years, with a mean age of 32 years.

Table 1: Dengue suspected and confirmed cases

No. Of suspected cases of Dengue	Dengue NS1 ELISA Positive	Dengue antibody MAC-ELISA Positive
300	121 (40.33%)	121 (40.33%)

Table 2: Distribution based on duration of illness

Duration of Fever (121)		
<4 days	4-7 days	>7 days
28 (23.14%)	78 (64.46%)	15 (12.39%)

Clinical Presentation

All patients presented with high-grade fever (100%). Other common symptoms included headache (68%), myalgia (55%), rash (30%), and hemorrhagic manifestations such as petechiae and gum bleeding (15%). Thrombocytopenia (platelet count <150,000/µL) was observed in 52 (43%) patients, and 10 (8%) required platelet transfusions.

Graph 1: Gender distribution

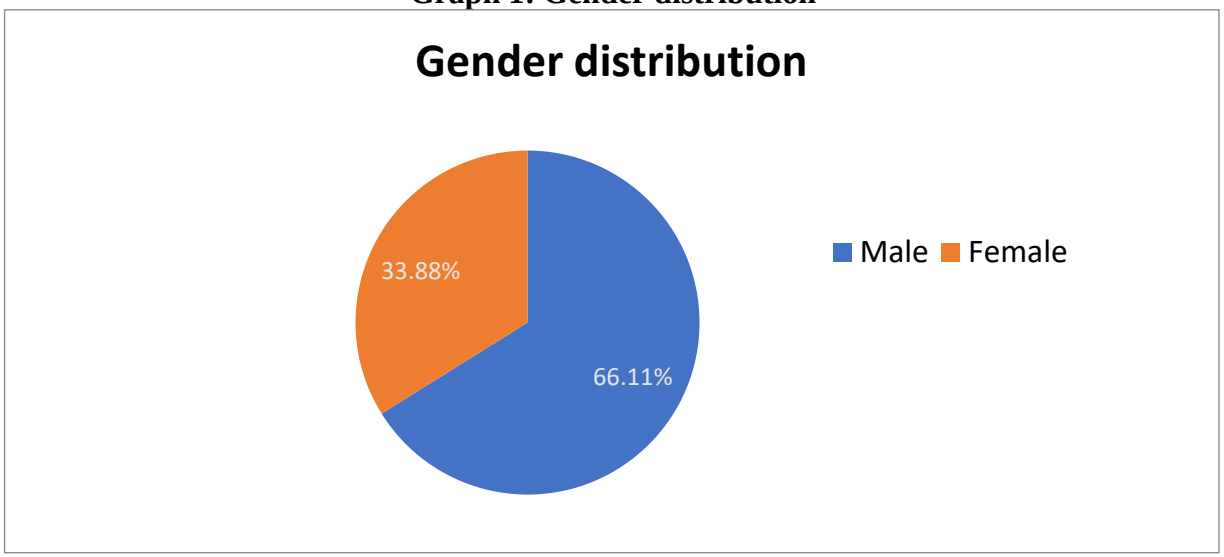

Table 3: ELISA results of Chikungunya IgM antibody

No. of samples	Positive	Negative
121	51 (42.15%)	70 (57.85%)

Table 4: Distribution based on duration of fever

Duration of Fever (121)	Dengue cases (121)	Chikungunya MAC-ELISA positive (51)	Chikungunya MAC-ELISA negative (70)
<4 days	28 (23.14%)	06 (11.76%)	22 (31.43%)
4-7 days	78 (64.46%)	39 (76.47%)	39 (55.71%)
>7 days	15 (12.39%)	06 (11.76%)	09 (12.85%)

The majority of patients (64.46%) presented with 4-7 days of fever duration.

Discussion

In this study of cross-reactivity or dual infection of Chikungunya and Dengue in an endemic area, several important findings were noted regarding demographic characteristics, clinical presentation, and laboratory investigations of the patients. Out of 300 suspected cases of Dengue, 121 (40.33%) were confirmed to have Dengue infection based on positive results for Dengue NS1 antigen and Dengue IgM antibodies (*Table 1*). This relatively high percentage underscores the endemic nature of Dengue in the region. Males were more commonly affected (66.11%), with a male-to-female ratio of approximately 2:1, aligning with other studies suggesting a higher prevalence of Dengue in males compared to females, though the reasons behind this gender disparity are not fully understood (*Graph 1*).

The age range of patients was 5 to 65 years, with a mean age of 32 years, indicating that Dengue affects individuals across a wide age spectrum in endemic areas. The majority of patients presented with fever lasting between 4 and 7 days (64.46%), which is consistent with the typical duration of acute febrile illness observed in Dengue infections (*Table 2*). Clinically, all patients presented with high-grade fever, as expected for Dengue, with other common symptoms including headache (68%) and myalgia (55%), characteristic of viral infections, particularly Dengue. Rash and hemorrhagic manifestations were noted in 30% and 15% of patients, respectively, with 43% experiencing thrombocytopenia. Although only 8% required platelet transfusions, this highlights the variability in disease severity and the need for careful monitoring.

The present study also highlights a substantial rate of cross-reactivity or dual infection between Dengue and Chikungunya viruses in an endemic area. Among the Dengue-confirmed patients, 42.15% also tested positive for Chikungunya IgM antibodies, suggesting the possibility of co-infection or

cross-reactivity (*Table 3*). This co-positivity rate underscores the challenges in differentiating between these infections based solely on clinical presentation and routine serological tests. The higher prevalence of co-positivity in patients with 4-7 days of fever duration aligns with the immunological response timeline, where IgM antibodies become detectable approximately 4-5 days post-infection [11] (*Table 4*). The overlapping symptoms and shared vectors facilitate simultaneous exposure to both viruses, increasing the likelihood of co-infections.

Comparative analysis with previous studies indicates variability in co-infection rates across different regions. For example, a study from New Delhi reported a 22% co-infection rate, which is lower than our findings but still significant.[8] Another study from Chennai reported a 15% co-infection rate, suggesting regional differences in vector prevalence and viral circulation.[9] In contrast, Singapore reported a much lower rate of 6%, likely due to effective vector control measures and differences in epidemiological patterns.[10] Majumder, et al., found a co-infection rate of 25% in Maharashtra, further illustrating the geographical variability in co-infection rates.[15]

The high co-positivity rate observed in this study could also be influenced by cross-reactivity in serological assays. ELISA-based tests, while useful for initial screening, may not distinguish between antibodies generated against different but antigenically related viruses.[12] This necessitates the use of more specific and sensitive diagnostic methods such as reverse transcription-polymerase chain reaction (RT-PCR) and plaque reduction neutralization tests (PRNT) for accurate differentiation.[13] Studies by Batista, et al.,[16] and Felipe, et al. (2018) have emphasized the importance of using RT-PCR in regions with high co-infection rates to avoid misdiagnosis.

The clinical management of Dengue and Chikungunya differs significantly. While Dengue can progress to severe forms requiring meticulous fluid management and monitoring for hemorrhagic complications, Chikungunya primarily necessitates symptomatic treatment for arthralgia [14]. Misdiagnosis can lead to inappropriate management and increased morbidity, as highlighted in the work of Furuya-Kanamori, et al., who reported cases of severe Dengue being managed as Chikungunya, resulting in poor patient outcomes.

Limitations

This study has certain limitations. Advanced diagnostic techniques like RT-PCR and PRNT were not employed due to resource constraints, which could have provided more definitive differentiation between co-infection and cross-reactivity. Additionally, the study was conducted over a limited period and at a single center, which may affect the generalizability of the findings.

Conclusion

The study reveals a high prevalence of cross-reactivity or dual infection between Dengue and Chikungunya viruses in the studied endemic area. These findings emphasize the need for implementing advanced and specific diagnostic methods to accurately distinguish between the two infections. Enhanced diagnostic accuracy is crucial for appropriate clinical management, reducing morbidity, and implementing effective public health interventions.

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