

Original article:

Dengue and Chikungunya co-infection associated with more severe clinical disease than mono-infection

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Abstract:

Background: Dengue and chikungunya infections appear to be increasing in India. While *Aedes aegypti* is the transmitting vector for both viruses and co-infection occurs in the same communities, studies on the clinical significance of co-infection are limited.

Materials and Methods: We conducted a retrospective case-control analysis of consecutive hospitalized patients presenting with febrile illness to the Sassoon General Hospital/BJ Medical College in Pune, India, who were screened for serologic evidence of dengue and chikungunya infection. Fischer's Exact test and Mann-Whitney test were used to compare mortality and morbidity between patients with dual and mono-infection.

Morbidity outcomes included lowest blood pressure (in first 5 days of admission), requirement for intensive care and mechanical ventilation, blood product transfusion requirement, as well as complete blood count.

Result: Co-infected patients had a higher overall mortality, than mono-infected patients (12% vs. 2%, p=0.04). Requirement for mechanical ventilation & number of blood units transfused were greater for co-infected vs. mono-infected patients (2% vs. 0%, p = 0.02 and median 6 vs. 4units, p=0.03, respectively).

Conclusion: Our study suggests that dual infection with dengue and chikungunya viruses is associated with more severe clinical disease, than mono infection. Further studies are required to determine whether our findings are associated with simultaneous or sequential co-infections, as well as to study the underlying pathogenesis of this association

Keywords: Dengue, chikungunya , co-infection, case control analysis, prognosis

Introduction

Dengue is an endemic arboviral infection which affects the tropical and the subtropical regions around the world, predominantly the urban and the semi urban areas. * Dengue viruses (DENV) cause 50 –

100 million annual cases of acute febrile illnesses worldwide, including more than 500,000 reported cases of the severe dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Since 1996, dengue has been widely prevalent in India and

reported from 18 States/Union territories, placing more than 450 million people at risk⁽²⁾. Chikungunya virus (CHIKV) infection has been identified in nearly 40 countries, and in 2008 it was listed as a US National Institute of Allergy and Infectious Diseases (NIAID) category C priority pathogen⁽²⁾. In India, it is estimated that more than 1.5 million people were infected with chikungunya⁽³⁾. It is known to cause large epidemic of hemorrhagic fever along with dengue virus causing fever, crippling joint pains, lymphadenopathy, and conjunctivitis.

Aedes aegypti is the principal vector in the urban transmission cycle of both DENV & CHIKV⁽¹⁾. In many areas of South Asia, the DENV and CHIKV epidemics affect the same communities, providing opportunities for *Aedes aegypti* to become infected with both viruses. This increases the risk of human co-infection, either through a single mosquito bite or via sequential infections.

Human co-infection with DENV and CHIKV have been reported in India since 1967⁽⁴⁾. Since DENV and CHIKV share a seasonal transmission cycle and have a number of similarities in clinical presentation, they difficult to distinguish without specialized serologic or molecular diagnoses.

While risk of severe disease, particularly DHF and DSS, have been associated with sequential infections with multiple dengue serotypes and a variety of immunologic mechanisms, there are limited studies examining whether co-infection or sequential infection with DENV and CHIKV are associated with more severe clinical disease^(5,6,7, 8,9,10,11). The pathogenesis of CHIKV is less well studied than DENV, with a variety of immunologic pathways implicated to be associated with clinical severity^(12, 14, 19, 20, 21). To address the question whether DENV and CHIKV co-infection or sequential infection was

associated with more severe disease than mono-infections, we conducted a study of sequential febrile patients presenting with clinical syndrome consistent with either disease in Pune, India.

Materials and methods

Clinical Samples

This was a retrospective case-control chart review which was conducted among consecutive patients hospitalized with severe febrile illness to the Sassoon General Hospital/BJ Medical College in Pune, India. This study was approved by the institutional ethical committee.

A total of 364 hospitalized patients were serologically screened for both DENV and CHIKV between September and December 2010. There was no sampling bias or any attempt to specially recruit patients for the study. The case records of the 364 hospitalized cases were analyzed for the clinical and the laboratory data.

Laboratory Diagnosis

DENV infection was defined as sera positive by IgM Capture ELISA using a commercial assay (NIV DENGUE IgM Capture ELISA Kit, Manufacturer: National Institute of Virology, Pune), with a reported sensitivity of 97.94 % and a specificity of 96.98 %⁽²⁶⁾. CHIKV infection was defined as sera positive by IgM Capture ELISA, using kits developed and evaluated by the US Centers for Disease Control (CDC), Fort Collins, USA (NIV Chikungunya IgM Capture ELISA Kit, Manufacturer: National Institute of Virology, Pune), with a reported sensitivity of 95 % and specificity of 98 %⁽²⁶⁾. Mono-infection was defined as a positive IgM assay for only one of these virus infections. Co-infection was defined as a positive IgM assay for both of these infections.

Clinical Assessment

Documentation of the basic demographic information and patient mortality among the study population was done. In addition, morbidity outcomes that were assessed included the nadir blood pressure within the first 5 days of admission, the requirement for intensive care unit admission, the requirement for mechanical ventilation, the number of blood product units transfused, as well as nadir laboratory findings within the first 5 days of admission including platelet count, total leukocyte count and total serum protein. Directly measured hematocrit or estimated hematocrit (i.e. hemoglobin g/dl X 3)⁽²⁷⁾, were also documented.

Data Analyses

Prevalence, median and range of demographic characteristics, as well as mortality and morbidity were compared between mono- and co-infected study subjects, using Fischer's Exact Test. The association of dual infection with increased mortality and morbidity outcomes was assessed using the Anderson-Darling Normality and Mann-Whitney Tests. (references?)

Results

Of the 364 consecutive hospitalized patients screened for both viral infections, 25 (6.8%) were IgM positive for both DENV and CHIKV. A total of 150 (41.2%) patients demonstrated serologic evidence of infection with only one of these viruses. Ninety-six (26.4%) were mono-infected with DENV and 54 (14.8%) were mono-infected with CHIKV. The remaining 189 (51.9%) patients were IgM negative for both viruses. The epidemic curves for DENV and CHIKV among hospitalized patients during this time period is shown in Figure 1.

Figure 2 shows the age distribution of hospitalized patients with DENV and CHIKV infection. The median age of mono- and co-infected patients was

similar (29 years and 26 years, respectively). Mono-infected patients with DENV were younger than mono-infected patients with CHIKV (median 25 years, range 9-76 vs. median 36 years, range 7-72)The gender distribution of mono-infected patients with mono-infection and co-infection was similar, with the majority of both groups male (64% vs. 68%, respectively). A higher percentage of mono-infected patients with DENV were male compared to mono-infected patients with CHIKV (67% vs. 59%)

Overall, 6 (1.6%) of the 364 patients died mortality was higher among patients admitted with serologic evidence of DENV-CHIKV co-infection vs. mono infection. Three (12%) of 25 co-infected patients died vs. 3 (2%) of 150 mono-infected patients (p-value = 0.039). A number of indicators of higher morbidity were more common among co-infected patients vs. mono-infected patients. Two (8%) of patients with co-infection required mechanical ventilation compared to none of the mono-infected patients (p-value=0.02). The median Units of Blood transfused to Co-infection patients were 6 (4-6 units) while that to mono-infection patients (150) were 4 (1-20 units), and this was found to be statistically significant (p-value 0.0319; p < 0.05) [Table 2].

Discussion:

Aedesegypti mosquito transmits both the Dengue and the Chikungunya virus. Chances of co-infection are higher if the mosquito carries both the viruses and therefore the problem of co-infection is more pronounced in areas where both these viruses co-circulate.

Clinical features are shared by both infections during the acute phase like common symptoms of both the diseases include fever, joint and bone pain, nausea, vomiting, headache, and fatigue. However, in our institution, all patients suspected of either Dengue or

Chikungunya are tested for both, hence eliminating the selection bias in our study.

Though symptoms of these diseases are common, their outcome differs. Chikungunya is mostly non-fatal while dengue may lead to severe complications including death. Thus co-infection may result in illness with overlapping signs and symptoms, making diagnosis and treatment difficult for physicians. Hence it is pertinent to address the issue of co-infections.

These viral infections are most common few months after the monsoon i.e. the peak of both co-infections and mono-infections was observed between first week of October and mid-November (according to the *Figure 3* of our study).

In our study we analyzed 175 patients out of which 150 patients were infected with either Dengue or Chikungunya and 25 were co-infected. According to our study, deaths due to co-infection are higher than mono-infection (12% v/s 2%, $p=0.04$). One of the study stated that individuals with *co-infection* appear to be more likely to suffer from complications and to have a higher risk of *death*^[31]. This data is significant since there is no specific treatment for these infections but early detection and access to proper medical care lowers fatality rate.

Also patients having co-infection have higher morbidity and it is significant from our study that there is greater requirement of mechanical ventilation and units of blood transfused in co-infected patients as compared to mono-infection patients (2% v/s 0%, $p = 0.02$ and 6 v/s 4 units, $p=0.03$, respectively). The hospital set up should therefore be well equipped for providing services of mechanical ventilation since the requirement may go up during a co-infection epidemic. As an increase in blood transfusions is

expected, the concerned authorities should be notified in advance.

From the clinical perspective, diagnosis of a co-infection should alert the physician to be more vigilant for complications. He should also frequently monitor the laboratory parameters, especially TLC (Total Leukocyte Count) since it has been shown in our study that patients with co-infection have higher total leukocyte counts than mono-infected patients.

Conclusion:

Our study suggests that dual infection with Dengue and Chikungunya viruses is associated with more severe clinical disease, than mono infection. Further studies are required which will help in eliciting the pathogenesis of this probable increased severity of co-infection.

Limitations:

The limiting factor of our study could be unavailability of RT-PCR diagnostic test in our institution because it is not a standard of care. Although studies using IgM antibodies to Chikungunya & Dengue for diagnosis of Dengue and Chikungunya co-infection have been conducted earlier⁽²⁹⁾.

Also our hospital being a tertiary care institute, some patients usually presented 5 days after onset of symptoms, thus limiting the use of RT-PCR.

Financial Disclosure:

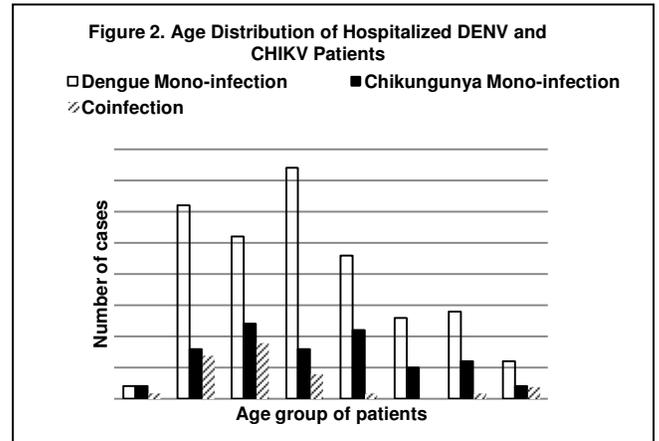
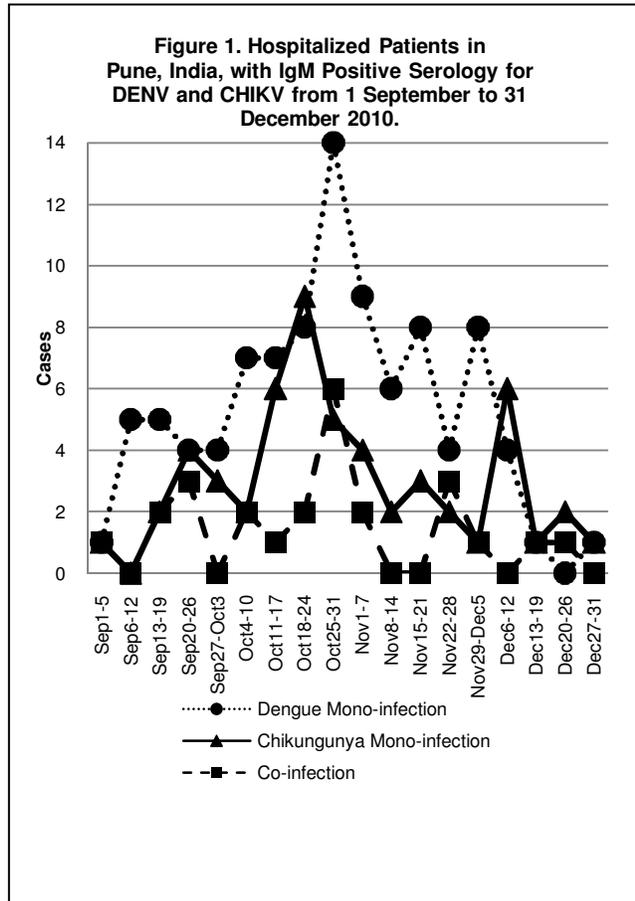
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Author contributions:

Among the masthead authors, this study was supervised by Robert C. Bollinger, RenuBharadwaj, Nikhil Gupte and Amita Gupta. Bhooshan S. Gandhi, KaushalKulkarni, ManasiGodbole, ShivaniKapur, PrajnaSatpathy, Shreya S. Dole, Akshay M. Khatri, Priyanka S. Deshpande, Fatema Azadcarried out the

data collection, analyses and prepared the manuscript. All the authors participated in these analyses and their interpretation, read and edited this manuscript, and decided to submit this manuscript for publication. The authors attest to the completeness

and accuracy of the data and the analyses. The corresponding author had full access to all the data and the final responsibility to submit for publication. **Competing interest:** There are no disclosures for the individual authors.



Abbreviations:

- DEN Dengue
- DENV Dengue Virus
- CHIK Chikungunya
- CHIKV Chikungunya Virus
- DHF Dengue Hemorrhagic fever
- DSS Dengue Shock Syndrome
- ADE Antibody Dependent Enhancement
- CMI Cell-mediated Immunity
- IFN Interferon
- TNF Tumour Necrosis Factor

	DENV Only	CHIKV Only	Co-Infection	Mono-infection	p-value*
Total Patients	96	54	25	150	
Deaths n (%)	1 (1%)	2 (4%)	3 (12%)	3 (2%)	0.04
Median Nadir Systolic BP** (Range)	104 (84-190)	110 (80-170)	110 (80-120)	110 (80-190)	0.54
Intensive Care Unit Admissions (%)	1(1%)	1 (1.9%)	1 (4%)	2 (1.3%)	0.37
Required Mechanical Ventilation (%)	0	0	2 (8%)	0	0.02
Required Blood Product Transfusion (%)	24 (25%)	4 (7%)	7 (28%)	28 (18.6%)	0.28
Median Blood Product Units Transfused (Range)	4 (1-12)	6.5 (2-20)	6 (4-6)	4 (1-20)	0.032
Median Hematocrit(%) Median (Range):	31.5 (22-49.5)	34.95 (15-54)	39.6 (27-48)	39 (15-49.5)	0.085

Table 1: Morbidity & Mortality of Hospitalized Patients with DENV and CHIKV Infection in Pune, India

RRV	Ross River Virus
ELISA	Enzyme Linked Immunosorbent Assay
HI	Haemagglutination Inhibition
RT-PCR	Reverse transcriptase- Polymerase Chain Reaction
DAMA	Discharge Against Medical advice
NIAID	National Institute of Allergy & Infectious Diseases, USA
NIV	National Institute of Virology, Pune

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