Short Report: A Comparative Study of Clinical Features between Monotypic and Dual Infection Cases with Chikungunya Virus and Dengue Virus in West Bengal, India

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Abstract. Chikungunya virus (CHIKV) and dengue virus (DENV) are circulating individually in the state of West Bengal, India. However, after 1965 the dual-infection caused by both viruses had not been recorded until 2010. In 2010, an investigation of the febrile cases was carried out to confirm the involvement of both viruses simultaneously. A total of 550 blood samples were tested for the detection of immunoglobulin M (IgM) antibody against both CHIKV and DENV. Serology by the enzyme-linked immunosorbent assay method confirmed that 131 (23.8%) and 104 (18.9%) patients had IgM antibody against CHIKV and DENV, respectively, whereas 68 (12.4%) had IgM antibodies against both CHIKV and DENV. Fever, joint pain, rashes, headache, myalgia, and nausea/vomiting are the common features in the case of both monotypic and dual-infection. Severe arthralgia and swelling of joints were common only in CHIKV-positive cases and abdominal pain was mainly associated with DENV infection. Diarrhea was reported only by the dual-infected patients (16.2%).

Arthropod-borne viruses or arboviruses are one of the major public health problems worldwide. Out of many arboviruses, chikungunya virus (CHIKV) and dengue virus (DENV) are the two most rapidly spreading arboviruses. The CHIKV belongs to the genus *Alphavirus* of the *Togaviridae* family, whereas DENV belongs to the family *Flaviviridae* and genus *Flavivirus*. To date, both CHIKV and DENV are co-circulating in India and Southeast Asia.¹ Both viruses are the RNA virus and the diseases caused by them are transmitted to humans by the vector mosquitoes *Aedes aegypti* and *Aedes albopictus*.² Both diseases have some common signs and symptoms that include fever, rashes, joint pain, nausea, headache, and vomiting.

In India, CHIKV was first recorded in West Bengal in 1963–65 along with the dengue outbreak.³ Chikungunya cases were not recorded in West Bengal after that outbreak, however until 1973, several outbreaks caused by CHIKV were recorded from other states of India.⁴ After that the virus disappeared from India.⁵ In 2005–2006, CHIKV outbreaks were reported from many states of India, including West Bengal.⁶

Dengue is one of the rapidly spreading infections affecting 50 million people per year.⁷ In India, DENV was first isolated in Kolkata in 1824,⁸ however the outbreak caused by DENV was first recorded in Kolkata in 1963.

Co-circulation of CHIKV and DENV is not uncommon in South-East Asia.^{9–11} In India, concurrent isolation of CHIKV and DENV had been reported since 1964 from different States.^{2,9} In 2010, a hospital-based study revealed co-circulation of CHIKV and DENV in some areas of West Bengal, India with high morbidity.

The aim of our work was to study the socio-demographic features of dual-infected cases, suffered from both CHIKV and DENV infection simultaneously and to compare the clinical features between the monotypic and dual-infected patients in West Bengal, India. For this purpose, in 2010, a study was conducted from the suspected cases, referred by the district health authority and by the clinicians of different

hospitals. Samples were collected from the patients admitted with high fever ($> 39^{\circ}\text{C}$) and any two of the following symptoms, i.e., rashes, joint pain, swelling of joints, nausea/vomiting, headache, myalgia, and retro-orbital pain. The local hospitals reported the absence of bacterial etiology and parasites in the blood samples. Informed consent was obtained from the patient or from the parents or legal guardians of minors before the collection of samples. A total of 550 samples were referred by the clinicians to our department for detection of CHIKV or DENV with detailed socio-demographic information and clinical history. Written consents were obtained before collection of the samples. Leukocyte counts of the patients were $3.5 \times 10^9/\text{L}$ – $5 \times 10^9/\text{L}$ and the platelet counts were $105 \times 10^9/\text{L}$ – $160 \times 10^9/\text{L}$. The sera were separated from the clotted blood samples and stored in aliquots at -80°C for further use.

All of the samples were subjected to an enzyme-linked immunosorbent assay (ELISA) test to detect the presence of immunoglobulin M (IgM) antibodies against both CHIKV and DENV by IgM antibody-capture (MAC)-ELISA kits. The kits were purchased from the National Institute of Virology, Pune, India.¹² Optical density (OD) was measured at 492 nm using an ELISA reader. The normal deviate test was performed to compare the data. The Z-values were calculated manually. The P value < 0.05 was considered significant.

Out of 550 samples, 131 (23.8%) and 104 (18.9%) samples were positive to IgM antibody against only CHIKV and DENV, respectively, whereas, 68 (12.4%) samples were positive to IgM antibody against both CHIKV and DENV. No cross reactivity was observed between the two viruses.

For the reverse transcription-polymerase chain reaction (RT-PCR) test, viral RNA was isolated from all the samples by using the Qiagen viral RNA isolation kit (Qiagen, GmbH, Hilden, Germany). The RT-PCR test was performed following the cost-effective RT-PCR method for detecting both CHIKV and DENV.¹³ The DENV typing was performed by using nested PCR with serotype-specific primers.¹⁴ Out of 550 samples tested, both DENV and CHIKV were detected in 24 samples; of which 18 samples contained DEN-2 serotype and 6 samples contained DEN-3 serotype. The IgM antibody against DENV or CHIKV was not detected in these 24 samples. The CHIKV RNA was detected in another seven

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TABLE 1
Demographic features of the IgM-positive cases in West Bengal, India in 2010

Variables		No. of cases (%)		
		Chikungunya (N = 131)	Dengue (N = 104)	Co-infection (N = 68)
Age groups (years)	Adult (≥ 16 years)	117 (89.3%)	59 (56.7%)	62 (91.2%)
	Children (0–15 years)	14 (10.7%)	45 (43.3%)	06 (8.8%)
	P value	—	—	—
Gender	Male	39 (29.7%)	40 (38.5%)	25 (36.76%)
	Female	79 (60.3%)	64 (61.5%)	43 (63.24%)
	P value	P = 0.03	P = 0.0188	P = 0.03
Place of residence	Urban/semi urban	98 (74.8%)	70 (67.3%)	42 (61.76%)
	Rural	33 (25.2%)	34 (32.7%)	26 (38.24%)
	P value	P = 0.001	P = 0.005	P = 0.012

samples that were positive by the ELISA method for DENV. No viral RNA was detected in the samples that were IgM positive against CHIKV by the ELISA method.

Demographic profiles of the IgM positive cases have been given in Table 1. Out of 68 IgM positive dual-infected cases, only six patients (8.8%) were ≤ 15 years of age. Adults were more affected by both viruses. However, the populations in different age groups are not uniformly distributed and hence the relative ratio of the children and adults in the dual-infected groups cannot be compared, and the result envisages only the tip of the iceberg. The highest number of co-infected cases was found in the age group of 31–40 years (27.9%) (Figure 1). The female/male ratio was 1.72:1, which is significantly high ($P = 0.03$). The females were much more affected than males because they reside in the house at daytime and may get exposed to the vector *Aedes* sp., which is domestic in nature and a day biter.^{15–17} No significant difference was observed between the residents of urban/semi-urban and

rural areas, although people in the urban/semi-urban areas were more affected by both monotypic and dual infection.

A comparison of clinical features is presented in Table 2. Fever is the most common feature in both single and dual infection, followed by joint pain, rashes, headache, and nausea/vomiting. Biphasic fever was found in all the dual infected cases. Swelling of joints and severe arthralgia are the common symptoms in the case of CHIKV infection, but was rare among the dual-infected patients. Most of the cases with only DENV infection were associated with abdominal pain, which was present in only one case with dual infection by both CHIKV and DENV. The most interesting observation made in this study was the clinical feature diarrhea, which was reported only by the dual-infected patients (16.2%). All the dual infected patients recovered quickly. In all cases the OD value of the Chikungunya IgM antibody was at least four times higher than the OD value of the dengue IgM antibody.

FIGURE 1. Age-wise distribution of the immunoglobulin M (IgM)-positive cases in West Bengal, India, 2010.

TABLE 2
Clinical characteristic of co-infected patients referred from different medical colleges and hospitals in West Bengal, India in 2010

Clinical features	Chikungunya cases (%)	Dengue cases (%)	Co-infected cases (%)
	(N = 131)	(N = 104)	(N = 68)
Fever	131 (100)	104 (100)	68 (100)
Joint pain	92 (70.2)	45 (43.3)	53 (77.9)
Rash	52 (39.7)	19 (18.3)	40 (58.8)
Headache	53 (40.5)	60 (57.7)	28 (41.2)
Myalgia/body ache	40 (30.5)	46 (44.2)	7 (10.3)
Itching	23 (17.6)	12 (11.5)	8 (11.8)
Nausea/vomiting	27 (20.6)	41 (39.4)	6 (8.8)
Joint swelling	85 (64.9)	4 (3.8)	8 (11.8)
Arthralgia/difficulty in movement	79 (60.3)	0	0
Abdominal pain	0	22 (21.2)	1 (1.5)
Retro-orbital pain/redness of eyes	0	8 (7.7)	4 (5.8)
Diarrhea	0	0	11 (16.2)

Regarding the monthly distribution of co-infected cases, the highest number of cases was found in the month of October (43.3%) followed by the month of November (31.3%) (Figure 2). The stagnant fresh water during the rainy seasons (June to September) favored the breeding of the vector mosquitoes. Therefore, the co-infected cases attained its peak in the month of October, which is the post-monsoon period.

In West Bengal, the first CHIKV outbreak was recorded during 1963 to 1965 in Kolkata, (formerly Calcutta) along with the outbreak of DENV. After 1965, CHIKV totally disappeared from this region. In 2006, after a gap of 40 years, the virus again reappeared.¹⁵ It has been observed by us that by 2010 CHIKV had gradually grabbed almost every district of this state by replacing the Asian genotype to Central/East African genotype (unpublished data).

The state of West Bengal is an endemic zone of DENV.⁸ Several outbreaks have been reported from this region.^{8,18} Although both viruses individually affected a large number of people of this state, after 1965, the dual infection caused by both viruses were recorded in 2010 from this region. The possible reason for dual infection may be because in West Bengal and in India the mosquitoes *Ae. aegypti* and *Ae. albopictus* are abundantly present and are also the vectors for CHIKV and DENV.¹⁹ *Aedes aegypti* are predominated mainly in the urban areas, whereas *Ae. albopictus* can survive in both rural and urban environments.²⁰ The vectors can carry both of the virus, which might have facilitated the spreading of the dual infection in both rural and urban regions.

Some man-made situations such as urbanization, industrialization, and deforestation result in vector shuffling in many

FIGURE 2. Monthly distribution of immunoglobulin M (IgM)-positive cases in West Bengal in 2010.

areas and raises the vector densities.¹⁸ The DENV can cause severe hemorrhagic illness and CHIK, although generally “benign,” but can cause severe neurological illness. Therefore, further epidemiological and virological investigations for both viruses are required, because these may create devastating effects, particularly in children and young adults who may not possess the CHIKV and DENV antibody.

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