



COINFECTION OF CHIKUNGUNYA AND DENGUE VIRUSES: A SEROLOGICAL STUDY FROM NORTHERN REGION OF BIHAR, INDIA

Bushra Manzoor	MBBS MD, Tutor, Department of Microbiology, DMC Darbhanga.
Manish Kumar Purbey	MBBS MD, Tutor, Department of Microbiology, DMC Darbhanga.
Dhirendra Kumar*	MBBS MD, Assistant Professor, Department of Microbiology, DMC Darbhanga. *Corresponding Author
Ram Shanker Prasad	MBBS MD, Associate Professor, Department of Microbiology, DMC Darbhanga.

ABSTRACT Introduction: Dengue and chikungunya (CHIK) infections has shown increasing trends in all parts of India. *Aedes aegypti* mosquitoes is the common vector for dengue virus (DENV) and CHIK virus (CHIKV). Transmission of both the virus is very common in areas where they cocirculate. Very few studies discuss the dengue- chikungunya coinfection from North Bihar region of India. The present study was undertaken to study the prevalence of dengue-CHIK coinfection in the North Bihar region of India. **Materials And Methods:** IgM antibody capture (MAC) ELISA for dengue IgM and CHIK IgM and ELISA for nonstructural protein1 antigen was performed on serum samples obtained from suspected patients. **Results:** Out of total 122 samples positive for DENV infection CHIK IgM antibodies were positive in 8 patients out of the total 122 cases. The clinical comparison showed that the coinfecting patients had fever in all cases while rash was seen in 72% cases of DENV. Arthralgia (76%) and thrombocytopenia was seen in significant number of coinfecting cases thus revealing overlapping nature of dengue-CHIK co-infection. **Conclusion:** Increase in the number of Dengue and Chikungunya infections and their cocirculation is an important public health concern which warrants the implementation of strict control measures.

KEYWORDS : *Aedes aegypti*, coinfection, ELISA, virology research and diagnostic laboratory

INTRODUCTION

Arthropod transmitted viruses, also known as arboviruses, are global threat especially in tropical and subtropical countries including India. These viruses are transmitted to human through blood sucking insect vectors commonly by mosquitoes and ticks. India has witnessed several outbreaks of arboviruses especially dengue and chikungunya in last few decades, making these viruses to gain a special attention. India is endemic for both dengue.

and chikungunya viruses, and presence of all the four serotypes of dengue virus have been reported from various parts of the country. Various clinical investigations from India have documented the dual infection with these viruses. However, the true disease burden of Dengue and Chikungunya dual viral infections is still not known because most of these studies involved a smaller patient group. *Aedes aegypti* is an important vector mainly found in tropical and subtropical areas across the world [1] and is implicated in the spread of several arboviruses; most important of them being dengue virus (DENV) and chikungunya virus (CHIKV). Dengue fever (DF) is a viral illness caused by a flavivirus and spreads by bite of *A. aegypti* mosquito. There are four serotypes of the virus referred to as DV-1, DV-2, DV-3, and DV-4. The name dengue originated from the Swahili word for “bone-breaking fever” or the word for “the walk of a dandier” in Spanish.[2] The spectrum of disease ranges from self-limited DF to more severe forms of dengue hemorrhagic fever (DHF) or dengue shock syndrome. The first major epidemic of the DHF occurred in 1953–1954 in Philippines followed by a quick global spread of epidemics of DF/DHF.[3] In India, the first confirmed outbreak occurred in Kolkata in 1963–1964.[4] Since then, there are many reports of dengue outbreaks from various parts of India. CHIK fever is a viral disease caused by an alpha virus that is also spread by bite of *A. aegypti* mosquito. The Chikungunya virus (CHIKV) belongs to the Togaviridae family and genus Alphavirus. It has a linear positive sense RNA genome of 1.8 Kb in length. Three genotypes (Eastern, Central South African [ECSA], West African and Asian) have been described for the Chikungunya virus. The name is derived from the Makonde word meaning that which bends up in reference to the stooped posture developed as a result of the arthritic symptoms of the disease.[5] CHIK first established its presence during a 1952–1953 epidemic outbreak in Tanzania.[6] In India, CHIKV was first isolated in Calcutta in 1963. The virus disappeared from our country after last reports from Maharashtra in 1973.[7] It then re-emerged in 2006 after a gap of 32 years and caused an explosive outbreak affecting 13 states.[2] Laboratory diagnosis of the two viral infections is done by virus

isolation, genome detection (RT-PCR) and antibody detection (IgM or IgG ELISA). In addition, the antigen detection (NS1 ELISA) is also being used for diagnosis of Dengue viral infection. In Asia, the CHIKV-affected areas overlap with DENV-endemic areas [8,9] and provide opportunities for mosquitoes to become infected with both viruses. Both the diseases have some common signs and symptoms which include fever with chills, swelling of major and minor joints with pain, difficulty in moving limbs, nausea, headache, and vomiting, and sometimes appearance of rashes.[10] In India, concurrent isolation of CHIKV and DENV had been reported since 1964 from different states.[9,11] There have been very few reports of coinfections from this part of the country in the past so we undertook this study to know the prevalence of dengue, CHIK coinfection in North Bihar and nearby districts.

MATERIALS AND METHODS

The study was conducted in Virology Research and Diagnostic Laboratory, Darbhanga Medical College (DMC) Darbhanga, Bihar during May 2019 to February 2020. Blood samples were collected from patients attending different wards in hospital or visiting the outpatient departments at DMC and Hospital, with typical clinical history of high fever (>39°C) with chills, rashes, joint pain, swelling of joints, nausea/vomiting, headache, myalgia, and retro-orbital pain. Approximately, 2–5 ml of blood was collected, serum separated, and subjected to ELISA. IgM antibody capture (MAC) ELISA was performed using kit provided by National Institute of Virology (NIV), Pune, for dengue on patients presenting with fever >5 days while ELISA for non-structural protein 1 antigen (fever <5 days) was performed (using Panbio Diagnostics kit, Germany) for patients presenting with fever of <5 days. CHIK IgM ELISA was performed on those samples in which request was made for testing CHIKV infection. In some samples both CHIKV and DENV were tested because requisition was made by the treating physician to check for both viruses. All tests were carried out following the manufacturer's instruction.

RESULTS

In the present study, total of 122 samples positive for DENV were tested for CHIK IgM antibodies out of which 8 samples (6.5%) were positive for CHIK IgM antibodies showing the prevalence of coinfection of DENV and CHIK infection of 6.5% [Table 1]. Gender distribution of the positive samples, showed that a higher percentage of mono infected patients with DENV were males (75%). However, there was no gender-based difference of infections among males and

females of coinfecting patients [Table 2]. Age-wise distribution of the DENV-infected and CHIKV-infected samples revealed that majority of the patients were in the productive age group of 21–30 years [Figure 1]. Clinical comparison of the cases showed that fever (100%) was present in all patients while rash was seen more commonly in dengue-positive (72%) patients as compared to those with CHIK (48%) and dengue–CHIK coinfection (24%). Furthermore, myalgia was seen more prevalent among dengue patients (64%) while arthralgia predominated among CHIK patients (76%).

Table 1 Prevalence Of Coinfection Of Denv And Chik Infections

CO INFECTION		CHIKENGUNYA IGM		TOTAL
DENV	POSITIVE	POSITIVE	NEGATIVE	
		8	114	122
TOTAL		8	114	122

Table 2 Gender Distribution Of Monoinfected Denv And Coinfected Patients

GENDER	COINFECTION		TOTAL
	YES	NO	
MALE	4	89	93
FEMALE	4	25	29
TOTAL	8	114	122

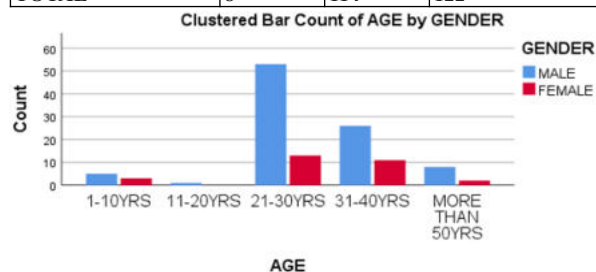


Figure 1 Age And Gender Wise Distribution Of Monoinfected Denv Patients

DISCUSSION

DF and CHIK infections are highly common in the northern region of India. Reports of co-infection are also showing an increased trend recently with overlapping signs and symptoms, making diagnosis and treatment difficult for physicians. As mosquitoes are abundantly present, they may become infected with both types of viruses and often get transmitted to human beings as coinfections. Serotyping of the virus infecting the population is important as it can help the clinician in proper treatment and management of the patient against complications like haemorrhages, ARDS, renal failure and arthritis. Hence, diagnosis of the type of infection can help the clinician in proper management of the patients during treatment and follow-up. There are few reports on dual infection, reported from various parts of India, showing patients co-infected with CHIKV and DENV [11,12]. In our study, coinfecting cases were not reported earlier but our study reported a prevalence of 6.5%. The prevalence of coinfection using serological methods has been reported in earlier studies by Kalawat *et al.* as 2.7%, [14] 2.8% by Omarjee *et al.*, [15] and 12.4% by Taraphdar *et al.* [12]. Maximum number of cases belonged to the age group of 21–30 years, in all types of infections as this group is maximally involved in outdoor activities, and being occupationally active has higher chances of exposure to arthropod-borne viruses. Similar trend was observed by Lall *et al.*, [16] Neeraja *et al.*, [17] Babaliche and Doshi, [18] and Chattopadhyay *et al.* [7].

The DENV was slightly more prevalent in males (75%) as compared to the females (25%) however the association was not significant with p value ($p=0.07$). Similar pattern was observed by other researchers. [21,22,23,24]. There was almost an equal representation of males and females among coinfecting cases.

Both types of infections were seen more during and subsequent to the monsoon months. The climatic conditions during this period favour vector-breeding places, thereby increasing the number of mosquitoes; resulting in a rise of dengue and CHIK. The reason may be due to prime occupation of the people being agriculture, and breeding of *A. aegypti* is highest during pre- and postmonsoon period. In dengue-positive cases, symptoms such as fever, rash, myalgia, and thrombocytopenia were more common as compared to CHIK in which arthralgia and

fever were the common presenting symptoms. Our clinical findings correlate with Babaliche and Doshi [18] and Londhey *et al.* [25]. The comparison of signs and symptoms showed that the coinfecting patients had fever in all cases while rash was seen in only 30% cases. Arthralgia and thrombocytopenia were also seen in a significant number of cases, thus revealing overlapping nature of dengue–CHIK coinfection.

Limitations Of The Study

The limitation of our study is the small sample size for dual infection cases. We would like to test our results with a larger sample size. Although RT-PCR is considered a standard criteria for confirmation of dual infections, we could not perform it as ours is a resource-limited setting. But we used ELISA kits recommended by NIV, Pune which have fairly good sensitivity and specificity for diagnostic purposes. In some other studies also, coinfection has been diagnosed on the basis of ELISA only. [11,14]

CONCLUSIONS

Repeated outbreaks of dengue, recent activity of CHIKV, and CHIKV/DENV coinfections in this region of North Bihar suggest that the endemicity of these viruses is in this region and. Thus, in clinically suspected cases of dengue or CHIK fever, it is advisable to test for both viruses as they cocirculate. The widespread emergence of DENV and exponential increase in CHIK cases warrant the need for more effective surveillance to monitor the spread of these deadly arboviruses so that timely control strategies can be implemented.

Conflicts Of Interest

There are no conflicts of interest.

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