



CO-INFECTIONS WITH CHIKUNGUNYA VIRUS AND DENGUE VIRUS IN AHMEDABAD, INDIA.

Clinical Microbiology

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ABSTRACT

Introduction: Dengue and Chikungunya both are endemic to urban and semi-urban areas of India. *Aedes aegypti* mosquitoes are common vectors for dengue virus (DENV) and chikungunya virus (CHIKV). Opportunities for their co-infections are increased due to the feeding behaviour of mosquitoes in areas where both viruses co-circulate. Clinical features of DENV and CHIKV are similar, so, CHIKV infections may go undiagnosed in DENV-endemic areas. Reverse transcription-PCR (RT-PCR) provides an earlier and more accurate diagnosis of the co-infections so that the Patient can be provided medications accordingly. **Aims & Objectives:** To determine the occurrence of Dengue-chikungunya co-infections in clinical samples received at Green Cross Genetics Lab. **Materials and Methods:** A retrospective data of dengue-Chikungunya coinfection detected by RT-PCR at green cross Genetics Lab was collected from June 2021 to December 2022. **Results:** Out of total 1430 samples from suspected patients for Dengue and Chikungunya infections, 212 samples were positive for DENV, 618 samples were positive for CHIKV. In addition to this, 6 samples were co-infected with both DENV and CHIKV. **Conclusion:** Concurrent infections may result in illness with overlapping signs and symptoms, making diagnosis and treatment difficult for physicians. Hence, in clinically suspected cases of dengue or chikungunya fever, it is advisable to test for both viruses by RT-PCR.

KEYWORDS

Dengue, Chikungunya, coinfection, RT-PCR

INTRODUCTION:

Dengue is an arbovirus belonging to the *Flavivirus* genus of the *Flaviviridae* family. DENV has four strains, referred to as DENV1–4 serotypes.^[1] Clinical manifestations of dengue are categorized into three phases: an initial febrile phase, a critical phase, and a spontaneous recovery phase. In the initial phase, the most commonly observed symptoms are headache, vomiting, myalgia, joint pain, and transient macular rash. Infection can progress to life-threatening conditions such as dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS).^[2]

Chikungunya virus (CHIKV) is an alphavirus. CHIK infection is often characterized by sudden onset of fever, headache, fatigue, nausea, vomiting, rash, myalgia, and severe and very painful polyarthralgia, which lasts for 1–10 days. However, arthralgia may persist for months to years.^[3,4]

Aedes aegypti mosquitoes are common vectors for dengue virus (DENV) and chikungunya virus (CHIKV). Opportunities for their co-infections are increased due to the feeding behaviour of mosquitoes in areas where both viruses co-circulate.^[5] Clinical features of both the viruses are similar, CHIKV infections may go undiagnosed in DENV-endemic areas.^[6]

Laboratory diagnosis of CHIKV and DENV infections can be confirmed by direct detection such as virus propagation, reverse transcriptase polymerase chain reaction (RT-PCR) or real-time RT-PCR. Specific antibodies such as IgM or IgG (antibody capture ELISA) is also used to detect infection as well but they depend on the time of collection and availability.^[4]

AIMS & OBJECTIVES:

To determine the occurrence of Dengue-chikungunya co-infections in clinical samples received at Green Cross Genetics Lab.

MATERIALS & METHODS:

The study was conducted in Green Cross Genetics Lab, Ahmedabad, during June 2021 to December 2022.

Study Design: In this retrospective study, Blood samples were obtained from patients attending various health units in Ahmedabad and other districts nearby with symptoms of Arboviral infections.

Molecular Analysis: Approximately, 2–3 ml of blood was received, plasma separated, and subjected to PCR. First, viral RNA was extracted using the ZYBIO-Nucleic Acid Extraction Kit (Magnetic Bead Method) following the manufacturer's instructions. The RT-PCR assay for detection of CHIKV and DENV was performed using the AmpliMole Dx™ DENV/CHIKV Multiplex Real Time PCR Kit following recommendations of the manufacturer. The reaction was carried out using Rotor gene-Q Real time PCR instrument. The processing of all samples included negative and positive controls was done to ensure reliability of the reaction.

RESULTS:

In the present study, we have included the samples for which requisition was made for testing both viruses. Out of total 1430 samples from suspected patients for Dengue and Chikungunya infections, 212 samples were positive for DENV. While 618 samples were positive for CHIKV [Table 1]. In addition to this, 6 were co-infected with both DENV and CHIKV.

Table-1: Total sample distribution of samples

	Total Samples Tested	Total Samples Positive
Dengue	1430	212
Chikungunya		618
Co-infections		06

Clinical comparison of the cases co-infected with both DENV and CHIKV was identified. All 6 patients had fever, headache, rashes, generalized body ache, joint pain, and low platelet & WBC counts. [Table 2]

Table-2: Clinical & Laboratory features of patients with co-infections

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age	53 years	51 years	13 years	42 years	40 years	58 years
Sex	Male	Male	Male	Female	Male	Male
Clinical features	Fever & severe joint pain	Fever with rigors, body ache	Fever with rigors, generalized body ache	Fever, rash & body ache	Fever & joint pain	Fever with body ache

Hb (gm/dl)	11.9	13.4	13.2	11.5	12.6	13.4
WBC count	3490	5000	7820	5000	4590	7500
Platelet count	120000	151000	295000	198000	150000	120000
Malarial parasite	Absent	Absent	Absent	Absent	Absent	Absent
Dengue NS1 by ELISA	Negative	Negative	Negative	Negative	Not done	Negative

DISCUSSIONS:

Dengue fever is highly endemic in urban and semi-urban areas of India. Chikungunya has similar clinical presentation as dengue.^[9] Co-infections with CHIKV and DENV occur in areas where these two viruses co-circulate. There are few reports on dual infection, reported from parts of India.^[6,7,10] As the vector for Dengue and Chikungunya infection is common, they may contain both viruses and may transmit both infections in humans.^[8]

A definitive diagnosis in acute phase of infection of Dengue can be done by Dengue NS1 antigen detection (Rapid or ELISA) and RT-PCR. In our study, we found 5 cases showing dengue NS1 antigen negative and RT-PCR as positive. RT-PCR is the most sensitive method to diagnose in acute phase of the Dengue infection.^[4] Though virus isolation is considered to be gold standard, it is time consuming.

Chikungunya detection by demonstrating IgM antibodies specific for CHIK in MAC-ELISA has its limitation due to cross-reaction with other Alphavirus antibodies. Therefore, RT-PCR is useful for confirmatory diagnosis prior to the appearance of IgM antibody.^[4]

Both types of infections were seen more during monsoon and post-monsoon period. The climate during this period favour vector-breeding, resulting in rise in dengue and chikungunya cases.^[8]

In dengue, symptoms such as fever, rash, body ache and thrombocytopenia were common, while in case of chikungunya fever with arthralgia were common symptoms. Our findings correlate with Kaur M^[7] and Londhey et al.^[11] The coinfecting patients had fever in all cases, while arthralgia and thrombocytopenia were seen in some of cases, thus revealing overlapping nature of dengue and chikungunya coinfection.

Drawback Of The Study:

The drawback of our study is the small sample size for coinfection cases. Therefore, we could not find prevalence at our region. We would like to test our findings with larger sample size.

CONCLUSION:

Concurrent infections may result in illness with overlapping signs and symptoms, making diagnosis and treatment difficult for physicians. Hence, in clinically suspected cases of dengue or chikungunya fever, it is advisable to test for both viruses by RT-PCR.

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