Section: Microbiology

Original Research Article

CHIKUNGUNYA CO-INFECTION AMONG DENGUE IGM POSITIVE CASES IN NORTH CHENNAI IN A TERTIARY CARE HOSPITAL

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Received : *10/07/2023*
Received in revised form : *12/08/2023*
Accepted : *28/08/2023*

Keywords:
Chikungunya, Dengue, IgM Antibodies.

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DOI: 10.47009/jamp.2023.5.5.111

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2023; 5 (5); 572-577

Abstract

Background: Dengue virus (DENV) and Chikungunya virus (CHIKV) are transmitted by Aedes mosquitoes, and the regions of CHIKV prevalence often overlap with DENV-endemic areas. It is challenging to differentiate clinical signs and symptoms of CHIKV infection from DENV infection. The co-circulation and co-infection of DENV and CHIKV in patients is quite common and reported from several countries, including India. The aim of this study is to diagnose chikungunya co-infection among dengue positive (IgM) cases by serological and molecular method (RTPCR). **Materials and Methods:** All blood samples tested positive for dengue IgM antibody in the Microbiology lab were included in the study for testing Chikungunya IgM antibodies using Pan Bio MAC IgM ELISA. Co-infected samples were first subjected to RT-PCR for DENV, then for CHIKV. **Result:** Blood samples of 136 patients who tested positive for Dengue IgM antibodies were tested for Chikungunya IgM antibody by MAC ELISA and 16.91% (23) samples were found to be positive. RT-PCR test performed for DENV and CHIKV detection in 23 co-infected samples (IgM co-infection) showed positivity for DENV in 30.43% (7/23) samples and CHIKV in 39.13% (9/23) samples. Among them, 17.39% (4/23) had co-infection with DENV and CHIKV. Among the 7 DENV RT-PCR confirmed positive samples (30.43%), the predominant serotype was DENV-2 (57.14%) followed by DENV-1 (43.86%). **Conclusion:** Both DENV and CHIKV are common illnesses of the monsoon which favour vector-breeding sites and the mosquitoes become infected with both types of viruses which are frequently transmitted to humans causing coinfections. The present study supports a better understanding of the different dengue serotypes circulating in the study population, and its co-infection with Chikungunya virus.

INTRODUCTION

Dengue fever is an acute viral illness caused by RNA virus of Flaviviridae family and spread by the bite of Aedes aegypti mosquito. Clinical features may range from asymptomatic fever to dreaded complications like hemorrhagic episodes, and circulatory shock. To reduce mortality, early diagnosis and treatment is necessary. Although dengue virus infections are usually self-limiting, dengue infection has come up as a public health challenge in the tropical and subtropical nations.[1]

Chikungunya fever is a viral disease caused by RNA virus of Togaviridae family which is spread by the bite of Aedes aegypti and Aedes albopictus mosquitoes. The name meaning, 'which bends up' refers to the stooped posture as a result of the arthritic symptoms of the disease. In Calcutta, CHIKV was first isolated in 1963. After being reported from Maharashtra in 1973, the virus was no longer present in our country. It reemerged in 2006 and set off an explosive outbreak that affected 13 states.[1,2]

Both DENV and CHIKV are transmitted by Aedes mosquitoes, and the regions of CHIKV prevalence often overlap with DENV-endemic areas. It is challenging to differentiate clinical signs and symptoms of CHIKV infection from DENV infection. The co-circulation and co-infection of DENV and CHIKV in patients is quite common and reported from several countries, including India. According to a hospital-based cross-sectional study in Odisha, from 2013,[3] 30%–40% of dengue-chikungunya coinfection cases were reported. In contrast to certain studies, which reveal a high

ISSN (O): 2687-5365; ISSN (P): 2753-6556

prevalence of severe symptoms and a poor clinical outcome among coinfected patients, others contend that neither the clinical outcome nor the symptoms were made worse by the coinfection. Serological analysis used to detect the concurrent spread of both viruses in humans, provide no information on whether either virus was still infecting people. Unavailability of viral specific diagnostic tools complicates medical management strategies and hence clinically viable and easily available molecular markers are the need of the hour. While pre-existing immunity towards CHIKV does not appear to worsen reinfection by another CHIKV lineage, heterotypic DENV reinfection can increase the risk of severe clinical outcome resulting from antibody dependent enhancement of infection. Hence, periodic seroprevalence surveys are important to evaluate immunity in a population to facilitate appropriate public health interventions.

MATERIALS AND METHODS

Ethical Considerations

Ethical clearance for the research study was obtained from the Institutional Ethics Committee (IEC), Government Stanley Medical College, Chennai.

Aim: The aim of this study is to diagnose chikungunya co-infection among dengue positive (IgM) cases by serological and molecular method (RTPCR).

Study Place: Departments of Microbiology, General Medicine and Paediatrics, Government Stanley Medical College, Chennai.Study design: A cross-sectional study was conducted from September 2022 to December 2022.

IgM ELISA for detection of anti‑dengue and anti‑chikungunya antibodies:

All blood samples tested positive for dengue IgM antibody in the Microbiology lab were included in the study for testing Chikungunya IgM antibodies. Approximately 2–5 ml of blood was collected, serum separated from whole blood by centrifugation from patients with suspected dengue, and subjected to Dengue IgM antibody capture (MAC) ELISA using Pan Bio kit. CHIK IgM ELISA test was performed on all dengue IgM positive samples by Capture (MAC) ELISA using Pan Bio kit. Serum samples were stored at − 20 °C. The optical density (OD) was measured at 450 nm, and the units of antibody concentration and cutoff values calculated as described by the manufacturers instruction.

Molecular analysis:

The molecular detection of CHIKV and DENV was done through RT-PCR. Co-infected samples were first tested for DENV, then analyzed for CHIKV. RT-PCR is in vitro nucleic acid amplification for the detection and quantification of DENV RNA and CHIKV RNA.

It contains reagents and enzymes for the specific amplification of the conserved region of the Dengue viral genome and Chikungunya viral genome, and for the direct detection of the specific amplicon in FAM channel. In addition, it contains an internal control amplification system to identify possible PCR inhibition and RNA purification efficiency. External positive control provided is used as both qualitative and quantitative to determine the amount of viral load. Primers used for the detection of DENV are,

Primer	Sequence (5′–3′)	Genome position	Expected size (bp)
DEN-F	5′-TCAATATGCTGAAACGCGCGAGAAACCG-3′	134–161	–
DEN-CR	5′-TTGCACCAACAGTCAATGTCTTCAGGTTC-3′	616–644	511
DEN1-R	5′-CGTCTCAGTGATCCGGGGG-3′	568–586	482
DEN2-R	5′-CGCCACAAGGGCCATGAACAG-3′	232–252	119
DEN3-R	5′-TAACATCATCATGAGACAGAGC-3′	400–421	290
DEN4-R	5′-CTCTGTTGTCTTAAACAAGAGA-3′	506–527	392

Similarly, specific primers were used for the detection of CHIKV
CHIKF — 5′-ACCGGCGTCTACCCATTCATGT-3′, nt10237-10258 and
CHIKR — 5′-GGGCGGGTAGTCCATGTTGTAGA-3′, nt10544-10566.

RESULTS

Table 1: Age and Gender distribution of study population (n=136)

Study group	Age group	n=136	Male	Female
Children (up-to 12 years)	1- 6 years	11 (8.1%)	5(45.45%)	6(54.55%)
	7-12 years	25(18.4%)	18(72%)	7(28%)
Adults (>12years)s	13-25 years	50(36.8%)	17(34%)	33(66%)
	26-50 years	43(31.6%)	15(34.89%)	28(65.11%)
	51-75 years	7(5.1%)	5(71.42%)	2(28.58%)
	Total	136(100%)	60(44.12%)	76 (55.88 %)

Table 2: Dengue and Chikungunya IgM Capture Elisa Results (N=136)

	Samples Tested (N=136)	
Results	Dengue IgM Elisa	Chikungunya IgM Elisa
Positive	136	23 (16.91%)
Negative	-	113(83.09%)

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Total	136	136(100%)

Table 3: Age and Gender distribution of Dengue and Chikungunya IgM co-infected population (n=23)

Study group	Age group	(n=23)	Male	Female
Children (up-to 12 years)	1- 6 years	2(8.7%)	2(100%)	-
	7-12 years	4(17.4%)	-	4(100%)
Adults (>12years)	13-25 years	10(43.5%)	4(40%)	6(60%)
	26-50 years	7(30.4%)	3(42.85%)	4(57.1%)
	TOTAL	23(100%)	9(39.13%)	14(60.87%)

Table 4: Demographic Distribution In Co-Infected Patients (IgM Dengue & IgM Chikungunya Positives) (N=23)

Demographic Distribution in Co-Infected Patients (IgM Dengue & IgM Chikungunya Positives)	
Location	**Number (N=23)**
NORTH CHENNAI	19 (83%)
OTHER PART OF CITY	4(17%)
TOTAL	23(100%)

Table 5: Correlation Between Platelet Count & Co-Infection (n=23)

Correlation Between Platelet Count & Co-Infection (N=23)	
Platelet Count	**Number (n=23)**
>150000	4 (17.39%)
100000 TO 150000	7(30.44%)
500000 TO 100000	12(52.17%)
Total	23(100%)

Table 6: Distribution of DENV, CHIKV and Co-infection of DENV AND CHIKV by RT-PCR (n=23)

RT-PCR TEST	DENV	CHIKV	Both DENV and CHIKV POSITIVE (CO-INFECTION)
Positive	7(30.43%)	9(39.13%)	4(17.39%)
Negative	16(69.57%)	14(60.87%)	

Table 7: Distribution Of Dengue Serotypes (n=7)

Dengue Serotypes	Number (n=7)
DENV 1	3 (42.86%)
DENV 2	4(57.14%)
Total	7(100%)

Blood samples of 136 patients who tested positive for Dengue IgM antibodies were tested for Chikungunya IgM antibody by MAC ELISA. In this study, we found that, among 136 dengue IgM positives, 36.8% (50/136) were in the age group of 13-25 yrs, of which females were more, about 66% (33/50), 31.6% (43/136) were in the age group of 26-50 yrs, 18.4% (25/136) were in the age group of 7-12 yrs, 8.1% (11/136) were in the age group of 1-6 yrs and 5.1% were in the age group of 51-75 yrs [Table, Figure 1]. Among 136 tested for Chikungunya IgM, 16.91% (23) samples were found to be positive [Table, Figure 2] , in which 43.5% (10/23) were in the age group of 13-25 yrs, 30.4% (7/23) were in the age group of 26-50 yrs , 17.4% (4/23) were in the age group of 7-12 yrs, 8.7% (2/23) were in the age group of 1-6 yrs [Table, Figure 3]. Majority of co-infected patients ie.83% (19) were from North Chennai (Table,Fig 4). Of these 23 co-infected patients with DENV and CHIKV, 17.39% (4/23) had platelet count >1.5 lakhs,30.44% (7/23) had 1-1.5 lakhs and 52.17% (12/23) with 50,000-1 lakh [Table 5].

All the patients had fever less than 7 days. RT-PCR test was performed for DENV and CHIKV detection in 23 co-infected samples (IgM co-infection). Out of 23 samples, DENV was detected in 30.43% (7/23) and CHIKV was detected in 39.13% (9/23) . Among the 16 positives of DENV and CHIKV, 17.39% (4/23) showed both DENV and CHIKV [Table 6, Figure 5].

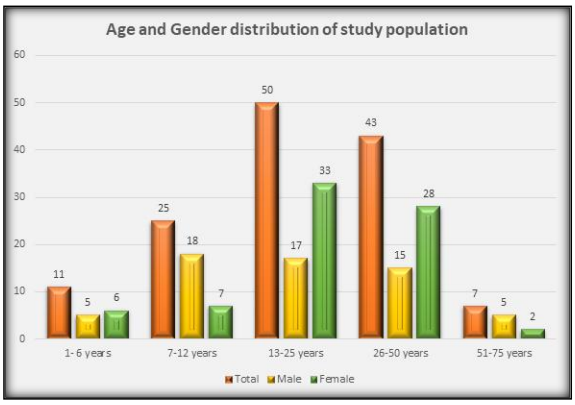

Figure 1: Age and Gender distribution of study population

Among the DENV RT-PCR confirmed positive samples(n=7,30.43%), only two serotypes DENV-1 and DENV-2 were detected. No infection with DENV-3 and DENV-4 serotype was found in the study.

Our study shows DENV-2 (n=4, 57.14%) was the predominant serotype followed by DENV-1 (n=3, 42.86%) [Table 7, Figure 6].

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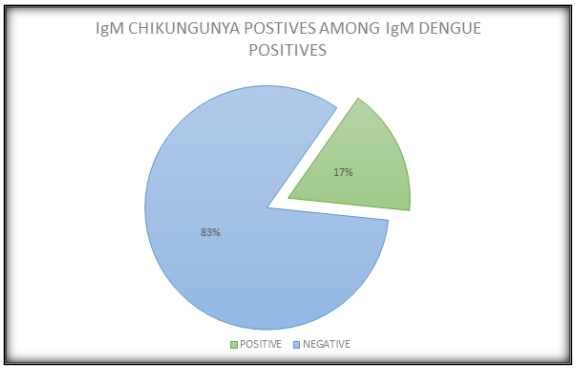


Figure 2: IgM Chikungunya Postives Among IgM Dengue Positives (N=136)

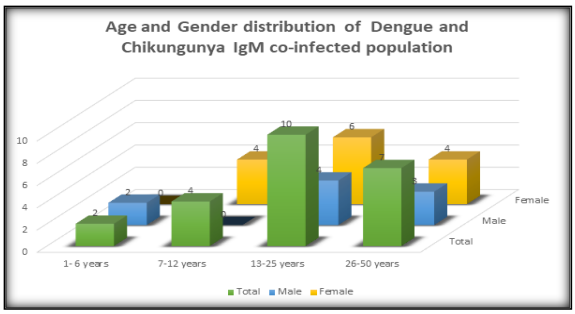


Figure 3: Age and Gender distribution of Dengue and Chikungunya IgM co-infected population

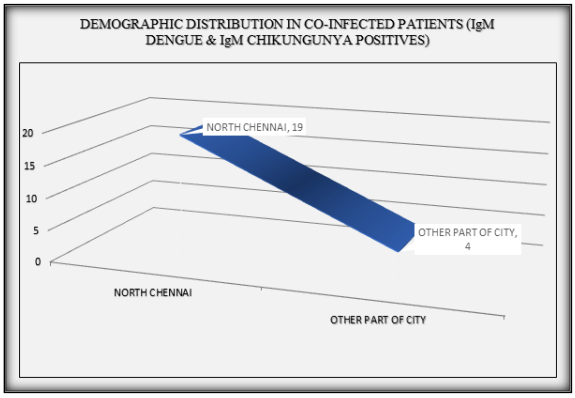


Figure 4: Demographic Distribution In Co-Infected Patients (IgM Dengue & IgM Chikungunya Positives)

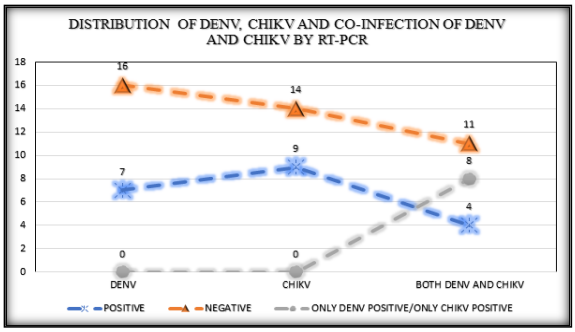


Figure 5: Distribution Of DENV, CHIKV And Co-Infection of DENV AND CHIKV RT-PCR Results

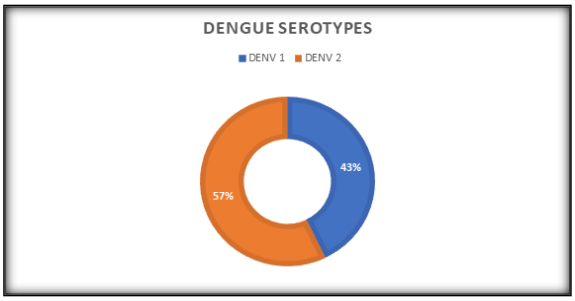
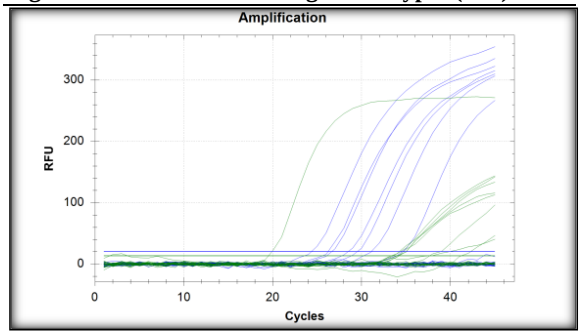
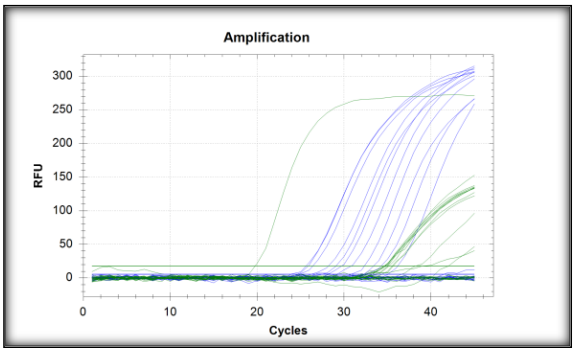


Figure 6: Distribution of Dengue Serotypes (n=7)



DENV RT-PCR



CHIKV RT-PCR

DISCUSSION

Few cases of CHIKV and DENV co-infection are reported, especially in people residing in endemic CHIKV and DENV regions. Nimmannitya et al. reported 4 co-infected cases out of 150 patients with either dengue or chikungunya (2.6%) in 1962 in Thailand.[8]

In a South Indian study, in 1964 out of 372 individuals chikungunya-dengue co-infection was detected in 2% of individuals with dengue-like-illness with 3 positives for DENV-2.[9] Despite the continued endemicity of CHIKV and DENV in Africa and Asia, studies documenting co-infection with chikungunya and dengue in Thailand, India ,and Myanmar were followed by the absence of reports of chikungunya-dengue co-infection for more than 30 years.[10-14]

Again co-infections with chikungunya and dengue were discovered in 2006 in Madagascar,[15] Sri Lanka,[16] India,[17] and Malaysia.[18] Clinical symptoms associated with CHIKV and DENV infections are similar in patients, as Asian countries have reported co-infection with CHIKV and DENV. Some information regarding co-infection is based on

ISSN (O): 2687-5365; ISSN (P): 2753-6556

the serological analysis of patient samples, however it provides scant information regarding active infection. In this regard, in-house designed primers for RT-PCR-based technique provided a varied yet commendable diagnosis of CHIKV and DENV as well as co-infections of both viruses.

In our study conducted in North Chennai, 16.91% (n=23) of the samples were positive for both Dengue IgM antibodies and Chikungunya IgM antibodies while Kaur M et al, reported 9.54% co-infection in Punjab,[2] Taraphdar D et al study,2012 reported 12.4% co-infection in West Bengal,[24] and Kalawat U et al, reported 2.7% co-infection in South India.[22] In our study, we found that 17.39% (4/23) of the samples were positive for both CHIKV as well as DENV by RTPCR in North Chennai, suggesting the co-existence of these two viruses in these patients. Study results were almost concordant to Afreen N et al,[20] 2014 Dec who reported 10% coinfection by RT-PCR and higher than the Khongwichit et al study,2022 who reported 0.4% co-infection by RT-PCR.[23]

The present study revealed the presence of two serotypes of Dengue virus, predominant being DENV-2 (57.14%) followed by DENV-1(42.86%). This is in concordant with Afreen N et al study (20) and Khongwichit et al study,2022 showing DENV-2 as predominant serotype,[23] but not coinciding with Neeraja M et al study showing DENV-3 and DENV-4 as predominant types.[21]

Although the pathogenicity and viral loads of the CHIKV strains obtained during our study have not been determined, their clinical spectrums are extremely similar and they can co-exist in the same host. The current study highlights the potential for misdiagnosis of chikungunya infections when there is concurrent background dengue transmission (and vice versa). This may hinder epidemiological understanding of both diseases and also have a significant impact on the clinical profile and prognosis of infected patients.

CONCLUSION

Dengue and Chikungunya fever are globally important arboviral diseases. The diseases are spread by same mosquito species, Aedes aegypti as the primary vector and Aedes albopictus as the secondary vector. It can be challenging to distinguish between the two illnesses clinically because they share many common clinical symptoms such as high-grade fever, rashes, nausea, headaches, and body discomfort. Chikungunya virus infection is not usually lethal, although it can induce neurological and ocular symptoms with restricted joint movements whereas Dengue Fever has bone pains and myalgia without restriction of movements. Both forms of illnesses are more common during and after the rainy season which favour vector-breeding sites and the mosquitoes can become infected with both types of viruses and thereby frequently causing coinfections .

Prevention of dengue and chikungunya virus disease depends on community-level mosquito control programs to reduce vector densities, and personal protective measures to decrease exposure to infected mosquitoes.The type of virus with which the patient is infected (either DENV or CHIKV) is significant because it can aid the physician in the choice of right therapy of the patient to prevent complications such as haemorrhages, ARDS, renal failure, and arthritis. The present study supports a better understanding of the different dengue serotypes circulating in the study population, and its co-infection with Chikungunya virus. It clearly suggests that the molecular analysis of the samples provide more reliable, accurate, and sensitive results when compared to the results obtained from the serological testing of the samples alone.

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ISSN (O): 2687-5365; ISSN (P): 2753-6556