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Emerging Co-Infection Patterns of Dengue and Chikungunya Virus in A Tertiary Care Hospital in Maharashtra, India

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A B S T R A C T

The healthcare system in India faces formidable challenges with mosquito-borne viral diseases. The tropical climate twined with dense population serves as a breeding ground of the *Aedes* mosquito, the vector for the communicable diseases, dengue and chikungunya. The Government of India has a standard protocol for treatment of Dengue but same must also be available for chikungunya. Cases become complicated by underdiagnosed co-infections i.e. when both the viruses are present in the patient and either both or any one goes unnoticed. Prevalence of such cases are on the rise as we have found from the patients visiting our hospital. This research specifically targets the western Maharashtra region, aiming to find the serological prevalence and pattern of dengue and chikungunya co-infections in comparison to mono-infections. Successful outcomes from this research may help to redesign the healthcare protocols and contribute significantly to redefine diagnostic strategies, to address the impact of dengue and chikungunya in western Maharashtra. This period of study was from May 2022 to April 2023. Blood samples (~2-5ml) were received in the Viral Research and Diagnostic Laboratory, Dept. of Microbiology from cases suspected of dengue or chikungunya. The patients' serum was tested using the ICMR-NIV provided IgM Capture ELISA kits for dengue/chikungunya. The records of the reports and patient details from VRDL, Dept. of Microbiology was collected and analyzed. Data analysis was performed using MS-Excel to prepare figures and tables. During the study period, a total of 1624 samples were tested for dengue/ chikungunya and coinfection. Out of 1624 samples tested, 271 samples were tested for dengue and chikungunya coinfection. Out of the 271 samples tested for coinfection, 27 (9.96%) were found to be positive. Among the 27 coinfected patients' sample, 11 were found to be male (40.74%) and 16 were female (59.26%). The age group of 31-40 years showed the highest coinfection rate (37.03%). Month wise data showed the highest coinfection rate in the month of August (18.5%) i.e., in monsoon, which was a demographic observation. Clear guidelines must be laid out to create awareness amongst patient population, to increase the number of testing for DENV and CHIKV. Strict surveillance must be ensured for high-risk population with special emphasis on the monsoon months. Detailed studies must be performed with coinfected patients to understand the nature of viral coinfection and the possibility of any new emergent/mutant virus.

Introduction

The study of Dengue and chikungunya co infection, both transmitted by Aedes mosquitoes, present substantial challenges to the healthcare system. These viruses have showcased a phenomenal rise in spreading infections primarily over the last few decades, contributing factors being but not limited to climate change, changes in patterns of human establishment, lack of systematic SOPs and guidelines. While both the diseases are individually capable of causing fatality, chances of any co infections might demand comprehensive attention due to their prevalence and potential complications (Cecilia, 2014). Both the diseases present symptoms in early stages like high fever, rashes, arthralgia and joint pain and in case of con infections sometimes the symptoms are more severe and prolonged.

However, laboratory testing and confirmation have revealed that it is extremely essential to not only distinguish between the two diseases and identify co infection cases if any to initiate faster and correct treatment. Studies have reported the compounding effect of underdiagnosed chikungunya in dengue infected patients, intensifying the healthcare burden (Deshkar *et al.,* 2018). Dengue virus (DENV) and chikungunya virus(CHIKV) are enveloped, positive-sense, single-stranded RNA viruses sharing common mosquito vectors, namely *Aedes aegypti* and *Aedes albopictus* (Bula *et al.,* 2022). Efforts have been taken to ascertain the seroprevalence of acute dengue and chikungunya virus infections, as well as their co-infection among patients presenting with clinical symptoms (fever, rash, arthralgia, hemorrhagic manifestation (Badoni *et al.,* 2023). The pathogenesis, transmission and underlying mechanisms of DENV and CHIKV can potentially become targets for intervention and therapeutics (Dawood *et al.,* 2021). Due to the high incidence, complications and higher mortality rate associated with dengue fever, symptomatic patients typically undergo testing solely for DENV, with chikungunya viral infection being considered only in rare instances (Dinkar *et al.,* 2018).

In a study it was seen that out of 200 suspected cases, 34 (17%) had a positive dengue serology, 77 (38.5%) had a positive chikungunya serology and 29.9% of the cases were positive for both dengue and chikungunya infection (Abhishek *et al.,* 2019). Prior DENV infection followed by subsequent CHIKV infection in the human hepatic cells showed the enhanced replication of CHIKV which can have detrimental effect on the liver. Viral propagation due to comodulating of DENV or CHIKV can affect the severity is the disease. These findings highlight the need for treating patients who have both dengue and chikungunya coinfection appropriately for areas where both viruses are common. Therefore, it is imperative to conduct investigations for both viruses, particularly in endemic areas (Taraphdar *et al.,* 2022). Recognizing the critical importance of accurate diagnosis and treatment is necessary as some studies have shown higher number of deaths due to co-infection when compared to mono-infection (Gandhi *et al.,* 2015).

A limitation of similar studies lies in less number of patient size and small population (n) due to which the gross effect and the extent of coinfections often goes unnoticed. Effective patient management and surveillance is essential in areas with high infection rates. It is essential for healthcare authorities to implement vector control and awareness programs. During monsoons when mosquito bite cases are on the rise tests for both the viruses must be made mandatory. It is essential to establish prompt pathogen diagnosis and a correlation between the severity of the disease and either a single or multiple infections (Deeba *et al.,* 2016).

The present study is aimed to understand the serological prevalence of dengue and chikungunya co- infections within the western Maharashtra region. comparing it with the prevalence of mono-infections was conducted.

Materials and Methods

The study was conducted in the Virology Research and Diagnosis Laboratory (VRDL), Dept. of M i c r o b i o l o g y of a Tertiary care teaching hospital in western Maharashtra from May 2022- April 2023. This was an observational study.

As per inclusion criteria our study population comprised patients suspected of dengue and chikungunya who were admitted in the in-patient department (IPD) or had visited the outpatient department (OPD) in a tertiary care hospital of western Maharashtra were included in the study.

Inclusion Criteria

i) All blood samples (~2-5ml) from suspected patients received in the Viral Research and Diagnostic Laboratory (VRDL) for dengue- chikungunya IgM ELISA testing.

Exclusion criteria

(i) Patients who refused to give samples in the OPD or IPD.
(ii) Blood samples which were lysed or damaged during collection/transportation.

Method: The serum from the samples were tested using the kits, IgM Capture ELISA for dengue/chikungunya provided by the ICMR-NIV (Vaghela Unnati *et al.,* 2022; Cheesbrough *et al.,* 2005).

Test Principle

The precoated Elisa plates containing the antihuman IgM (µ chain specific) to capture the IgM antibodies against dengue/chikungunya in the patient's serum. In the next step, Dengue antigen is added which binds to captured human IgM in the sample. Any unbound antigen has to be removed by washing. In the subsequent step biotinylated Flavivirus anti DEN monoclonal antibodies were added followed by avidin HRP. The chromogenic substrate (TMB/H2SO4) was added and the reaction was stopped using the stop solution i.e. 1N H2SO4. The optical density was measured at a wavelength of 450nm.

Test Protocol: Dengue/Chikungunya IgM ELISA

- ✓ Selection of sample and preparation of the test protocol on ELISA sheet provided with each kit.
- ✓ Wash buffer preparation by diluting 20x the wash buffer with distilled water to attain 1:20 concentration
- ✓ ELISA micro well strip was washed for 3 times and tapped dry.
- ✓ Positive and negative controls were added in to well A and B (50 µl in each well)
- ✓ Addition of 200 µl of sample diluent +2µl of sample to each well from C. (Incubate at 37°C,60min)
- ✓ Wash buffer was used to wash the strips 5 times and tapped dry on tissue paper
- ✓ Addition of 50µl of dengue/chikungunya antigen to each well (incubate at 37°C, 60mins) followed by repetition of step 6.
- ✓ Addition of 50µl of dengue monoclonal antibody to each well (incubate at 37°C, 60mins) followed by repetition of step 6.
- ✓ Addition of 50µl of avidin-HRP to each well followed by incubate at 37°C for 60mins
- ✓ TMB substrate was added to each well (100 µl) and incubated for 10 min in dark at room temperature
- ✓ Stop solution (100µl) was added to each well after 10mins.
- ✓ Absorbance was measured at 450nm immediately.

Data collection and processing

The details regarding the age, gender, date of testing was taken from the patient records in the VRDL Laboratory along with month wise occurrences.

Statistical Analysis

Data analysis was performed using MS-Excel to prepare figures and tables. Chi Square test was performed. P-value <0.05 was considered statistically significant.

All patients' information was kept confidential.

Results and Discussion

In this study, the total sample size (n) was 1624. Out of 1624 samples, 1239 samples were tested for dengue of which 284 (23%) were found to be positive. For chikungunya mono-infection, 385 out of 1624 were tested and 48 (12.4%) were found to be positive. Out of 1624 samples tested, 271 samples were tested for both dengue and chikungunya seropositivity and 27 (9.96%) were found positive for both. (Table 1) while analyzing the positivity rate in both the genders with respect to coinfection, it was found that there was a slightly higher positivity in case of females (59.26%) than in males (40.74%) (Table 2).

Many clinical symptoms in dengue and chikungunya are similar like fever, headache, myalgia etc. We found that there is no significant (*P*-value < 0.05) difference in the symptoms faced by either of the two gender (table 3). In age wise distribution (Fig1) the positivity rate of coinfection was found to be high in the age-group of 31-40 years (37%). The number of positive cases in the age category 21-30 years was higher at 23.2% for dengue whereas age group 31-40 years had positive cases higher at 35.4% for chikungunya. It was further noticed that the highest number of cases were in the month of august (18.5%) (Fig 2).

The present study aims to understand the coinfection dynamics of dengue virus (DENV) and chikungunya virus (CHIKV) which belong to the arbovirus family. Co

infection dynamics studies reveal information about viral replication and dominance i.e cases where competitive dominance over the host cell leads to dominance established by one virus over the other (Sastry and Bhat, 2018). The results reveal certain critical findings in patients with coinfection. However, co modulation dynamics may be influenced by associated factors such as age, immune exposures, comorbities etc. Investigations have revealed a coinfection rate of 9.96%. One of our findings also suggests females having higher rate (59.26%) of the coinfection cases as compared to males (40.74%). Whether this is related to any biological, behavioral and healthcare related issues requires further exploration.

The demography of the study population is in the areas of western Maharashtra which receives heavy rainfall and is densely populated. Our study has revealed that the maximally infected age-group is 31-40 years (37.03%). The finding correlates with Taraphdar *et al.,* (2012) who also reported that coinfected cases were highest in this age group. [(14)] August was found to be critical month with highest number of infections at 18.5% which is similar to a study done by Turuk *et al.,* (2021). The study correlates with the behavioral pattern of mosquitoes as throughout India, mosquito borne infections rise during the rainy season (Abhishek *et al.,* 2019; Turuk *et al.,* 2021).

On comparison with the study by Singh *et al.,* (2018); Dawood *et al.,* (2021) and Kaur *et al.,* (2019) their coinfection rate aligned closely with our finding. Singh *et al.,* (2018) reported a co-infection rate of 10.74%, Dawood *et al.,* (2021) observed a slightly higher rate of 11.7% (Dawood *et al.,* 2021; Dinkar *et al.,* 2018; Abhishek *et al.,* 2019). The reports of Kaur *et al.,* (2019) showed 9.4% coinfection rate.

These differences may be due to demographic and experimental variations, differences in the evolving patterns of arboviruses in the prevalence of dengue and chikungunya coinfection with time.

The present study finding is in concurrence with those of Singh *et al.,* (2018); Dawood *et al.,* (2021) and Kaur *et al.,* (2019) contribute to the growing rates of coinfection in patients suffering from both dengue and chikungunya.

The importance of this study lies in the fact that the possibility of viral comodulation in the affected patients cannot be ignored. Earlier studies of viral comodulation by Taraphdar *et al.,* (2022) have suggested that coinfection might adversely affect patient's health and also increase the severity of the diseases manifold. Co-infection with Dengue virus (DENV) and Chikungunya virus (CHIKV) is in itself a rare scenario where two RNA virus co-exist in a single host or inside the same host cell. This may lead to viral RNA interactions which may lead to immune modulation of the host system. The resultant evolution of a new viral strain with different viral patterns may be due to indirect mutations of two co-existing RNA viruses (Taraphdar *et al.,* 2022).

Study by Meng *et al.,* (2022) have shown instances of mutation in CHIKV in coinfected patients during the dengue outbreak in 2019. According to molecular epidemiology, the chikungunya virus found in this study had adaptive changes that set it apart from monoinfected cases. The study also raises an alarming picture of a more potent and virulent CHIKV as a result of such mutations. Meng study has also cited incidences of coinfection in the Indian population (Meng *et al.,* 2022). Studies by Waever and Forrester say that direct genetic recombination may not be possible between DENV and CHIKV as they are from different viral families. However viral comodulation and replication may increase chances of mutation leading to the formation of new species or quasi species considering the fact that both are RNA viruses (Weaver and Forrester, 2015).

The outcome of the study and its findings about coinfection patterns and coinfection dynamics of the Dengue and Chikungunya Virus belonging to the Arbovirus family expands beyond the boundary of the subcontinent, to tropical and subtropical regions like Africa, Brazil and South East Asia. The causative agents for both the diseases are mosquito vectors *Aedes aegypti* and *Aedes albopictus*. Our study results are in line with the findings of Kumar *et al.,* (2022) and Tandale *et al.,* (2009) which state that viral transmissions rise during the monsoons. Various studies have also reported that during this time coinfection rates have been reported to range from 7%-20% (Kumar *et al.,* 2022 and Tandale *et al.,* 2009). Our results try to establish the importance and necessity of a proper SOP and guideline for clinical management in case of any outbreak.

The viruses responsible for causing Dengue and Chikungunya are from the Flaviviridae and Togaviridae, family respectively. The mode of affecting the host immune system virus is not fully deciphered yet. The severity of infection and measure of viral load can potentially manipulate the hosts immune response (Saswat *et al.,* 2015).

Table.1 Number of tests done w.r.t the results obtained.

Sl.no.	Disease	Total	Positive	Negative	Equivocal
1.	Dengue	1239	284 (23%)	796 (64.2%)	159 (12.8%)
2.	Chikungunya	385	48 (12.4%)	299 (77.6%)	38 (10%)
3.	Coinfection	271	27 (9.96%)	--	--

Table.2 Seropositivity in males versus females

Sl. No.	Disease	Males (n=663)		Females (n= 684)	
		Positive (n=163)	Negative(n=500)	Positive(n=169)	Negative (n= 595)
1.	Dengue	144 (50.7%)	368 (46.23%)	140 (49.3%)	428 (53.77%)
2.	Chikungunya	19 (39.58%)	132 (44.14%)	29 (60.42%)	167 (55.86%)
3.	Coinfection	11 (40.74%)	--	16 (59.26%)	--

Figure.1

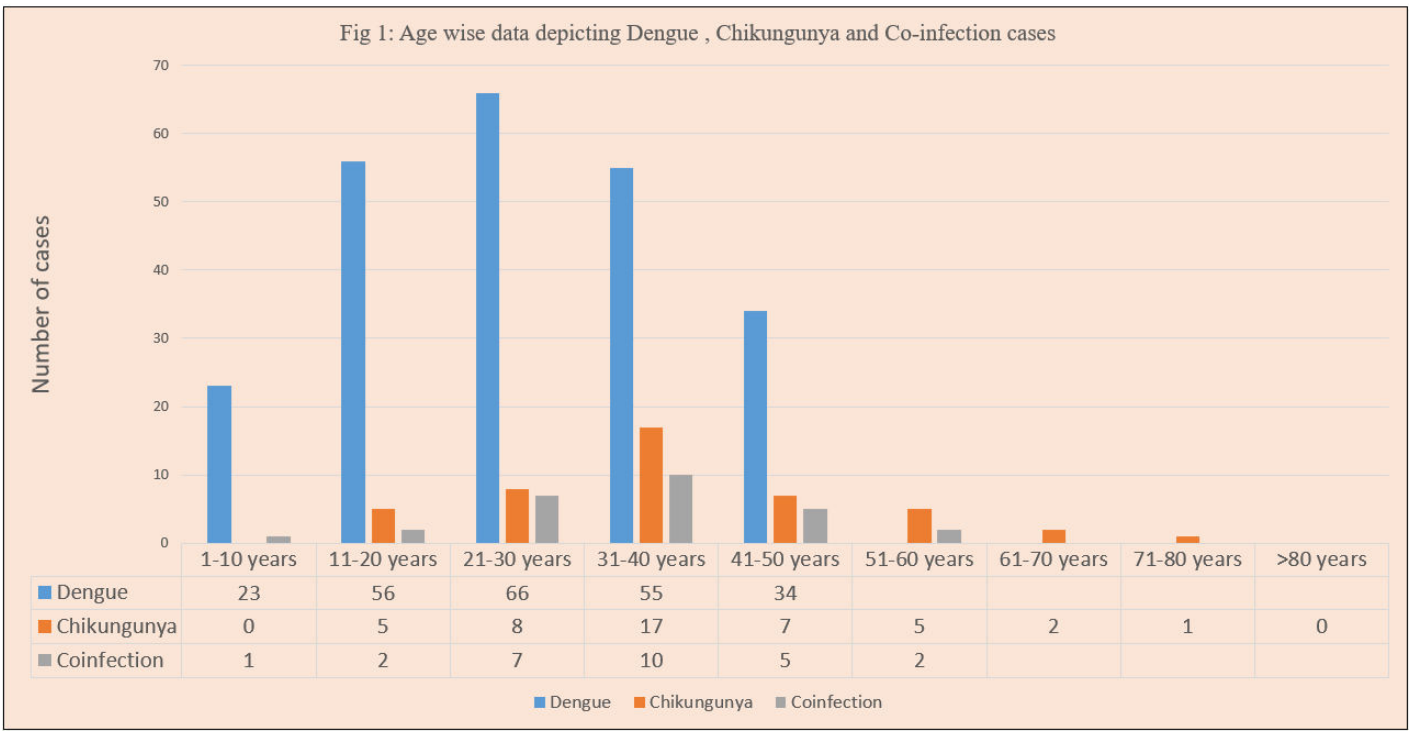
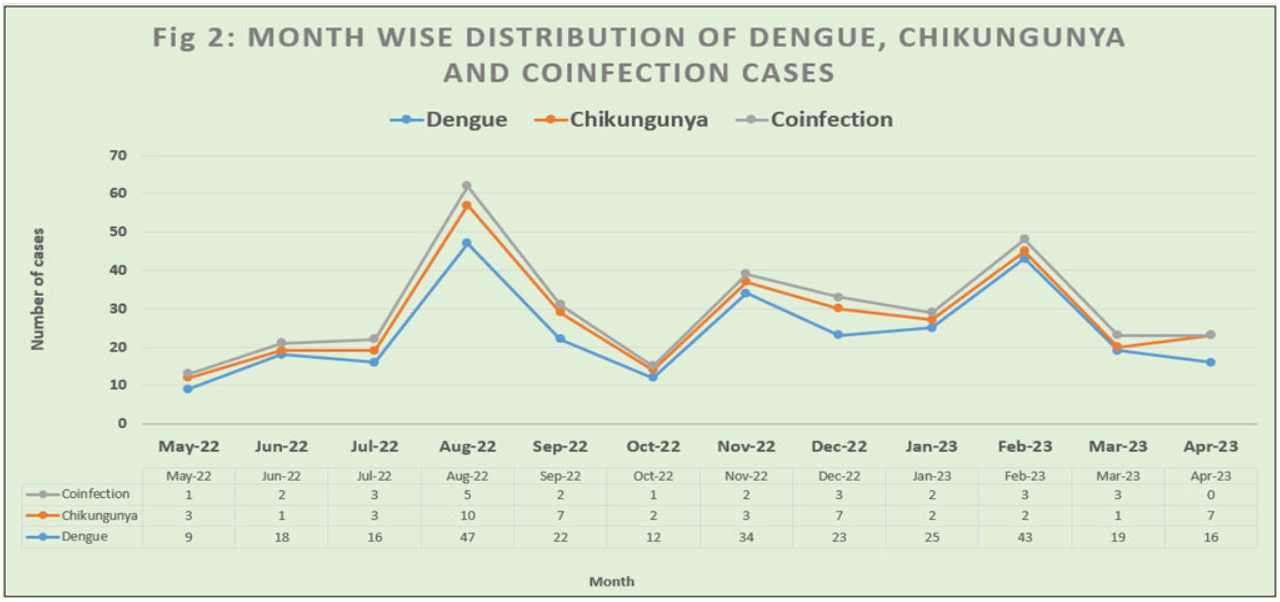


Table.3 Clinical presentation w.r.t gender

SL.No.	Symptoms	Male	Female	Total	χ^2 value	p-value
1	Fever	727 (95%)	832 (96%)	1559 (96%)	0.7797	0.37
2	Chill	719 (94%)	775 (89.3%)	1494 (92%)	0.1523	0.69
3	Rash	132(17.4%)	95 (10.9%)	227 (13.9%)	0.0003	0.98
4	Headache	570 (75.2%)	745 (85.4%)	1315 (80.9%)	0.0328	0.85
5	Arthralgia	632(83.4%)	780 (89.9%)	1412 (86.4%)	0.2519	0.61

Figure.2



Mutation is a distant possibility as both belong to different families co infection and may influence the immune system of the host affecting T cell response, cytokine production, innate immunity and overall disease outcome (Afreen *et al.,* 2014). Most of the rural areas in the western part of Maharashtra have limited set up and the diagnostic kits available are mostly rapid tests. These kits may not have the capability to detect co infections. This gap needs to be addressed to get correct health data related to co infections (Afreen *et al.,* 2014).

This study results show 9.96% coinfection rate of DENV and CHIKV in the study population. This can be due to a combination of several demographic conditions like heavy rainfall, deforestation, floods leading to increase in mosquito infested areas. Deeper studies of coinfected patients have NOT ruled out chances of viral genetic comodulation and mutations which may give rise to a more potent and virulent virus.

A clear and well drafted public health policy shall provide complete surveillance, reportion of increase in cases from affected zones and case reporting. Proper detection protocols and kits must be designed and implemented to detect both the pathogens. Often failures in testing may lead to underdiagnosed cases and underreporting of co infections.

This may seriously affect the disease outbreak which might change the epidemiology the region. Clear guidelines must be laid out for the general public with targeted surveillance throughout the year with special emphasis on the monsoon months. Detailed research must be carried out on coinfected patients to understand the nature of viral coinfection and the possibility of any new emergent/mutant virus.

In view of our study findings, it is important to assess for the risks associated with DENV and CHIKV coinfection and initiate proper management of coinfected patients as the presence of any of the virus or any comodulated or mutated form of the virus can affect the severity of the disease by changes in viral propagation. Since co-infection leads to a more severe clinical disease compared to mono-infection, underscoring the imperative for comprehensive strategies to manage and mitigate the impact of these arboviral co- infections in western Maharashtra is imperative.

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Author Contributions

Dr. Shreya Dutta: Conceived the original idea and designed the model, investigated analyzed the data and wrote the manuscript..; Dr. Pankaj A. Joshi: Software Validation, Data Curation, Supervision; Dr. Neeta P. Jangale: Writing-Review and Editing, Visualization and Project Administration.; Dr. Vanita A. Kulkarni: Resources, Draft Preparation.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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