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ABSTRACT

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COINFECTION OF DENGUE AND CHIKUNGUNYA VIRUSES: A SEROLOGICAL STUDY IN A TERTIARY CARE HOSPITAL IN WARANGAL, TELANGANA, INDIA

Background: All around India, Dengue and Chikungunya (CHIK) diseases seem to be rising. *Aedes aegypti* mosquitoes frequently transmit the CHIK virus and Dengue virus (DENV). Both viruses can spread together in regions where they cocirculate.

Objectives: The objective of the current investigation is to determine the seroprevalence of coinfection with Dengue and Chikungunya.

Methods: The present study was conducted at the Virus Research and Diagnostic Laboratory, Kakatiya Medical College, Warangal, Telangana, India, from January to December 2022. The district health authority and the admitted cases provided the samples. The patients had symptoms typical of CHIK and DEN infections, including high fever (>39°C), chills, rashes, joint pain, joint swelling, nausea, and headache, myalgia, and pain behind the eyes.

Results: Between January 2022 and December 2022, a total of 4892, 3344, and 2103 hospitalised patients underwent serological testing for the Dengue virus (DENV), the Chikungunya virus (CHIKV), and both Dengue and Chikungunya virus for co-infection, respectively. IgM Capture ELISA was used for all tests. 153 (7.8%) of the cases had both Dengue virus (DENV) and Chikungunya virus IgM positivity. (CHIKV). Dengue virus (DENV) monoinfection, or 703 (14.3%), denotes the presence of Dengue virus. 313 (9.3%) individuals tested positive for the Chikungunya virus, or mono-infected with the virus (CHIKV). Chikungunya IgM, Dengue IgM, and Co-infection

Conclusion: Dengue and Chikungunya virus co-infections are clinically more serious than a single infection. However, in endemic regions, particularly during the monsoon season, clinically suspected cases should be tested for both viruses. To

determine the severity and clinical result of co-infection, more thorough investigations are needed to analyse the pathophysiology and complications of co-infection.

Keywords: Coinfection, Dengue, Chikungunya, ELISA.

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INTRODUCTION / ВСТУП

Dengue virus (DENV) causes dengue, an important neglected tropical disease, and belongs to the family Flaviviridae, genus Flavivirus [1]. More importantly, it has been categorized as a "neglected tropical disease" [2]. They are both single-stranded, positive-sense RNA viruses called Dengue virus (DENV) and Chikungunya virus (CHIKV). DENV is a member of the genus Flavivirus, which has 5 recognised serotypes, and the family Flaviviridae. (DENV1–5). There are three recognised strains of the Togaviridae family member CHIKV (Asian-West African; East-Central; South African) [3]. Temperature, humidity, and rainfall are examples of environmental variables that may have an impact on the vector mosquito population. contribute to the rise in cases of dengue. Additionally, socioeconomic variables like age, sex, and race/ethnicity have been linked to a risk of DENV infections [4].

Each virus genome will be approximately 11 kb in length [5], three structural genes (C, prM, and E) encodes the DENV and seven non-structural (NS1, NS2B, NS3, NS4A, NS4B, and NS5) proteins [6]. Three structural genes (C, E1, and E2) encodes CHIKV and four nonstructural (nsP1–4) proteins [3]. Both DENV and CHICKV are arthropod-borne viruses (arboviruses) sharing a common vector: mosquitos of the Aedes genus, specifically *A. aegyptiand A. albopictus* [7]. Dengue is divided into two categories by WHO: Dengue (with or without warning signs) and severe Dengue [8]. Dengue has been known to exist in India for more than two centuries, but in recent years, the disease's epidemiology has undergone a significant transformation, with larger and more regular outbreaks being documented in both urban and rural areas. [9–11]. The co-circulation of four Dengue serotypes was first observed in Delhi in 2003, and despite a lack of regular virologic surveillance, each serotype has since been isolated from separate outbreaks in different countries [12–13]. Although there has been an increase in the proportion of severe cases, the majority of instances of the disease are still non-severe [14]. There is also doubt about the true disease burden, despite recent research findings that Dengue is likely to occur across the majority of the Indian subcontinent [15–16] and that only a small percentage of clinically evident cases are diagnosed and reported [17]. The amount of transmission at the population level has not been extensively studied in epidemiological investigations. The first Chikungunya outbreaks in India occurred in 1963, and further outbreaks occurred in a number of Indian states throughout the 1960s and early 1970s [18]. After appearing to have been absent for more than 30 years, the disease returned to Andhra Pradesh in late 2005. Throughout 2006, it expanded to other states in central and southern India. Observation based on emergency clinic reports for illnesses like Dengue and Chikungunya, where the extent of asymptomatic and mild infections varies, can be misleading and may not reflect the true degree of transmission.

Multiple outbreaks of Dengue have been reported throughout the country. As per the National Vector Borne Disease Control Programme (NVBDCP), by October 2022, >0.7 million (including 0.2 million in 2018–19) DENV and 58,000 (including 22,000 in 2018–19) CHKIV cases were documented in India [19–20].

Additionally, it is often impossible to differentiate between dengue, Chikungunya, and other febrile illnesses without a sufficient amount of serologic and virologic tests. As a result, observational data may be dependent on significant revealing predispositions in environments where such tests are not standard procedure. Seroprevalence investigations are anticipated to accurately assess and describe the level of transmission in such situations.

Both viruses circulate in similar geographic regions. In non-endemic regions, travel-related infections are a significant thought for patients with a new travel history who present with fever.

Simultaneous disease with both infections, sent from either two different mosquitos or one dually tainted mosquito, is possible. For DENV, transmission has also been accounted for to happen by means of contaminated blood items, organ donation, and prenatal and/ or perinatal vertical transmission.

This study sought to ascertain the seroprevalence of acute Dengue and Chikungunya virus infections, as well as their co-infection, among patients presenting with clinical symptoms in Warangal, Telangana India because there is a lack of information about the co-infection of DENV and CHIKV in this area.

Material and Methods

The study was carried out in Viral Research and Diagnostic Laboratory (VRDL) Kakatiya Medical College, Warangal, Telangana. The district health authority and the admitted cases provided the samples. The patients had symptoms typical of CHIK and DEN infections, including high fever (>39°C), chills, rashes, joint pain, joint swelling, nausea, and headache, myalgia, and pain behind the eyes. Only those patients who tested positive for CHIKV and in whom the primary cause of stomach discomfort was DENV infection experienced severe arthralgia and joint edema. Laboratory request forms contain information about the patient's demographics, medical history, and pertinent clinical investigations (haematological and biochemical). A simple vacutainer was used to collect 5 ml of blood that was drawn aseptically by venipuncture, left to clot at room temperature, and then centrifuged for 10 minutes at 2000 rpm. After centrifugation, the separated sera were aliquoted into sterile 1.5 ml storage vials. To identify the existence of immunoglobulin M (IgM) antibodies against CHIK, DENV, and co-infection independently, all samples underwent an ELISA test (enzyme-linked immunosorbent assay). IgM Catch ELISA Manufacturer: National Institute of Virology, Pune performed each test. A specific IgM Catch ELISA for both infections—Chikungunya and dengue—was used to define coinfection. For further treatment and the implementation of preventive measures, online reports of the results were sent to the relevant District and State health authorities on a daily, weekly, and monthly basis in the format of the Integrated Disease Surveillance Project (IDSP). All the data was also uploaded to the National Institute of Epidemiology (NIE) portal.

The entire data were entered and cleaned in MS Excel before its statistical analysis. All the results are shown in tabular and graphical format.

Results

During January 2022 and December 2022, a total of 4892, 3344, and 2103 hospitalised patients underwent serological testing for the Dengue virus (DENV), the Chikungunya virus (CHIKV), and both Dengue and Chikungunya virus for co-infection, respectively. IgM Capture ELISA was used for all tests. 153 (7.8%) of the cases had both Dengue virus (DENV) and Chikungunya virus IgM positivity. (CHIKV). Dengue virus (DENV) monoinfection, or 703 (14.3%), denotes the presence of Dengue virus. 313 (9.3%) individuals tested positive for the Chikungunya virus, or mono-infected with the virus (CHIKV). Results of Chikungunya, Dengue, and Co infections are displayed in Figure 1. In Dengue majority of the cases were from the age group of 0–10 years (26.95%) followed by 11–20 years (17.40%). In case of Chikungunya majority of the cases were from the age group of 0–10 years (32.69%) followed by 11–20 years (23.45%) Figure 2 and 3. In Dengue a seasonal peak was seen in the months of August to December. In case of Chikungunya seasonal peak was seen in the months of August to December. Of the total number of affected cases, in Dengue 2109(50.34%) were males and 2080 (49.65) % were females. In case of Chikungunya of the total number of affected cases 1801 (59.41%) were males and 1901 (62.71) % were females.

Discussion

Dengue virus (DENV) and Chikungunya virus are mostly spread by the mosquito species *Aedes aegypti* and *Aedes albopictus*. (CHIKV). Both viruses can spread infection to vulnerable people as co-infections following a mosquito bite since they are frequently present in the insect at the same time. Because the two diseases are hard to distinguish from one another, their symptoms frequently overlap. Common clinical manifestations of Dengue and Chikungunya viral infections include high-grade fever, headache, nausea, rashes, and bodily pain. The most noticeable symptom of Chikungunya infection is the excruciating joint pain, which can occasionally last for several months to a year. The neurological and ocular symptoms of a severe Chikungunya virus infection. Thus, while Dengue fever can cause serious consequences, including death, Chikungunya viral infection is typically not lethal. Therefore, a sickness with overlapping symptoms could develop as a result of co-infection with the two viruses.

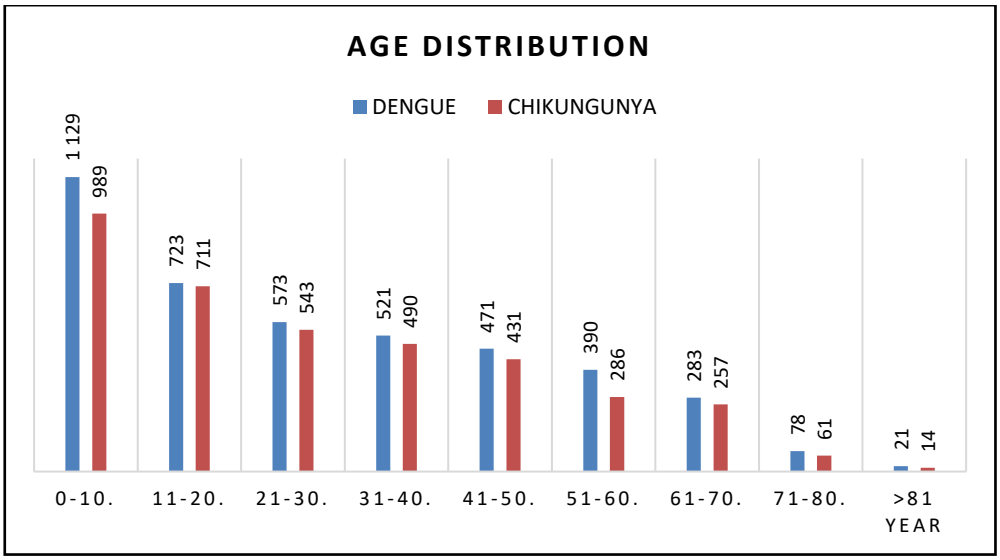

Figure 1 – Distribution by age wise of the Dengue and Chikungunya IgM positive cases

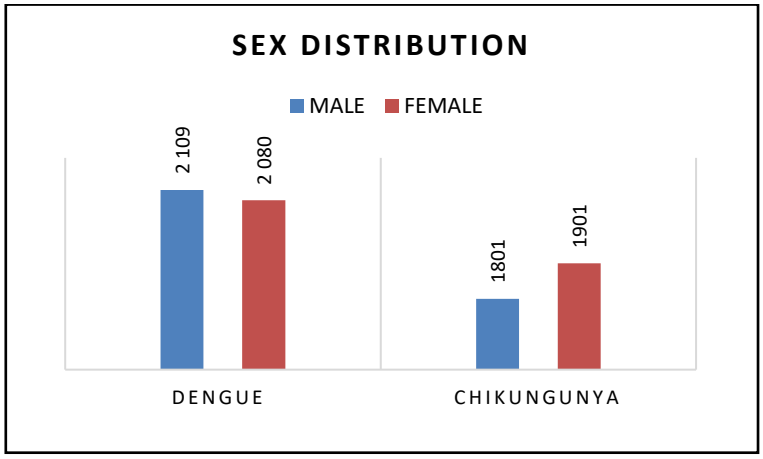

Figure 2 – Sex distribution of dual IgM positive cases

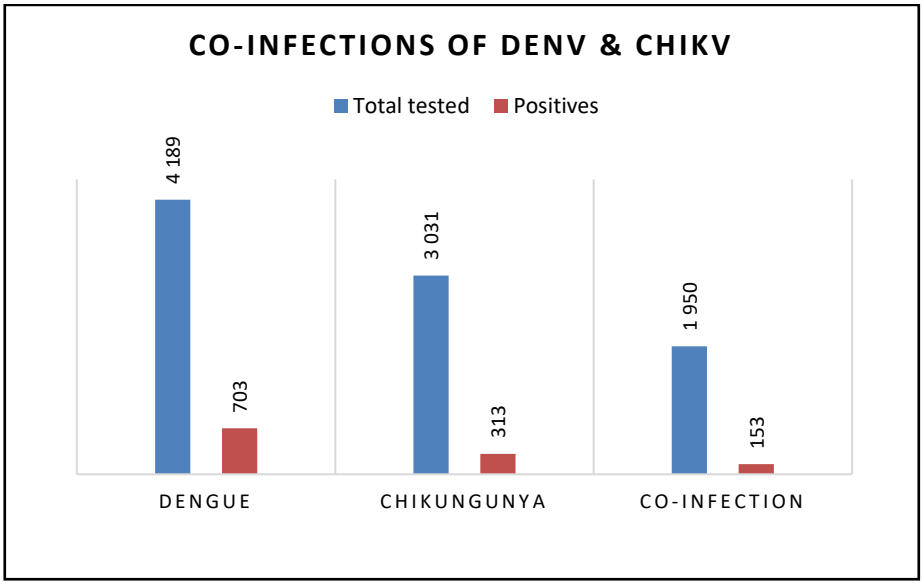

Figure 3 – Represents the Co infection of DENV and CHIKV

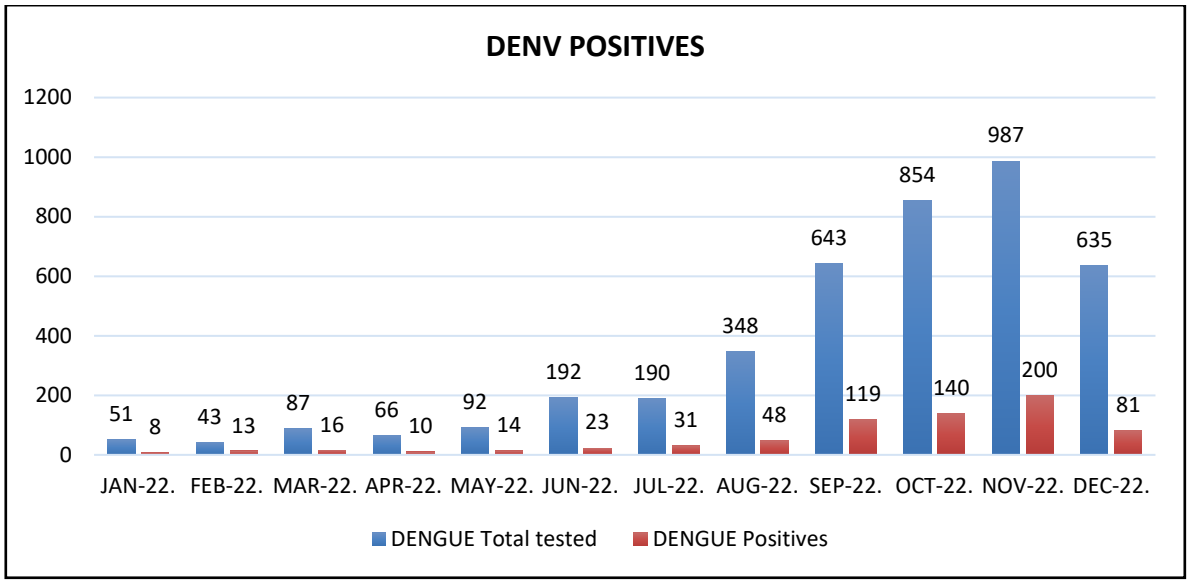

Figure 4 – Represents the Monthly distribution of Dengue, IgM positive cases

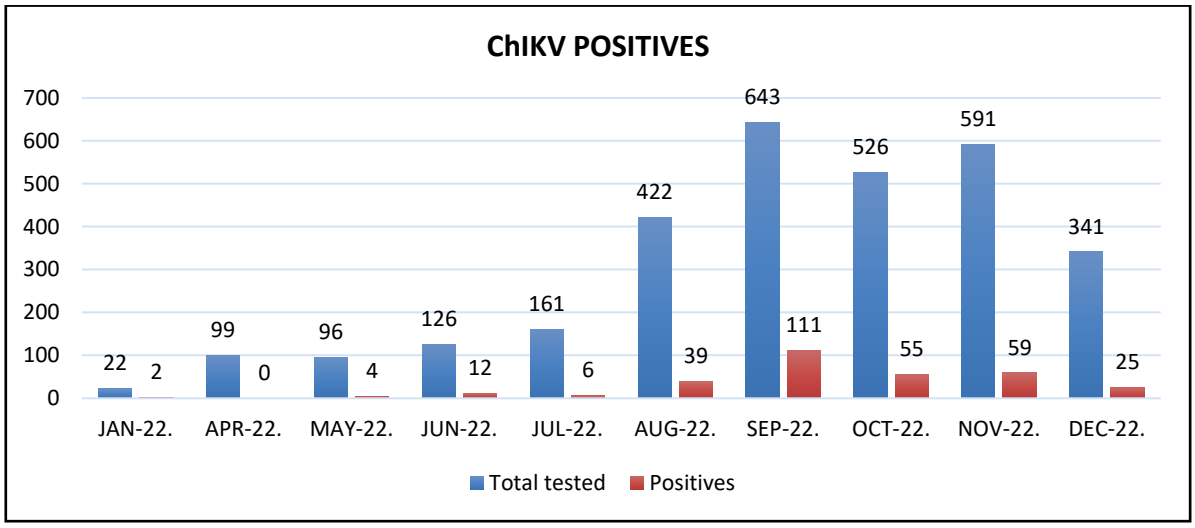

Figure 5 – Represents the Monthly distribution of Chikungunya IgM positive cases

IgM Capture ELISA was used for all tests. 153 (7.8%) of the cases had both Dengue virus (DENV) and Chikungunya virus IgM positivity. (CHIKV). Dengue virus (DENV) monoinfection, or 703 (14.3%), denotes the presence of Dengue virus. 313 (9.3%) individuals tested positive for the Chikungunya virus, or mono-infected with the virus (CHIKV). In another similar study conducted by Bodani et al 2023 DENV cases (79.5%) were more outnumbered than CHIKV (5.7%), and the co-infection of DENV-CHIKV was 1.4%. Another similar studies were reported from North-west, North, North-East, and central India. A comparison of these studies is given in Table 1.

The number of cases rises during and after the monsoon season because higher humidity extends mosquito life spans and warmer temperatures reduce extrinsic incubation time [29]. Previous research from India had noted a rise in cases throughout the monsoon season, with the biggest numbers occurring in various months [30–32]. In this study, the peak monsoon month for Dengue was November. In this study, the peak monsoon month for Chikungunya was September. Compared to 2.7% in a study by Kalawat et al., co-infection with Dengue and Chikungunya was discovered in 7.84% of cases in our investigation [33]. A Similar study conducted by Singh et al 2018 [34] in their study November was peak among the monsoon months. The number of instances increased from August to December while decreasing from January to June. Most studies observed this type of seasonal change because

Table 1 – Comparison of different North, northeast, and central India studies on DENV, CHIKV, and co-infection

Studies	DENV (%)	CHIKV (%)	Co-infection (%)
Kaur et al. [21]	68.9	34.0	9.5
Raina et al. [22]	7.8	0.6	6.2
Hisamuddin et al. [23]	40.0	40.0	9.0
Singh et al. [24]	84.2	4.9	10.7
Zelaya et al. [25]	49.0	29.0	10.0
Chahar et al. [26]	69.5	24.6	8.6
Prasad et al. [27]	75.0	6.0	19.0
Shrinet et al. [28]	20.7	28.3	22.6
Present Study	14.3	9.3	7.8

during the beginning of the rainy season, when infected vector mosquitoes are more prevalent because higher humidity lengthens their life span and higher temperatures decrease the extrinsic incubation time, transmission intensifies. The age range 0–10 years was the most afflicted, while older adults >81 years had a reduced incidence of cases in our study. Males were more likely to contract Dengue than females, although females were more likely to contract Chikungunya. This study's primary objective is to compare the serological results of Dengue and Chikungunya cases to those from earlier reference studies. A similar study was conducted by Bodani et al 2023 in case of gender distribution the male female ratio was higher in Clusters 1 and 4. Males have outnumbered females [35]. Another similar study by Oscar M. Vidal was done in 2020, in which Males were more likely to contract Dengue than females, although females were more likely to contract Chikungunya [36]. Many recent studies reported coinfection is associated with clinically severe disease leading to high mortality compared with Mon infection [37]. Therefore, further studies involving larger sample sizes in endemic areas are needed to better understand the clinical and biochemical profile in dual viral infections.

CONCLUSIONS / ВИСНОВКИ

Dengue and Chikungunya virus co-infections are clinically more serious than a single infection. However, in endemic regions, particularly during the monsoon season, clinically suspected cases should be tested for both viruses. To determine the severity and clinical result of co-infection, more thorough investigations are needed to analyse the pathophysiology and complications of co-infection. This would make it easier to determine the true cost of coinfection with Dengue and Chikungunya. Effective and timely management can help predict epidemics and contain them.

PROSPECTS FOR FUTURE RESEARCH / ПЕРСПЕКТИВИ ПОДАЛЬШИХ ДОСЛІДЖЕНЬ

In order to effectively manage Dengue vector mosquitoes, it is necessary to use chemical, biological, and ecological methods of management. To slow the spread of Dengue, focus on areas where people and mosquitoes come into close contact with one another. It has been demonstrated that the number of people infected with Dengue virus may be decreased through the use of insecticide–treated curtains and novel mosquito traps. Surveillance is an additional constituent for the prevention of dengue, as it furnishes the requisite data for evaluating the level of risk and directing the programme. The gathering of information concerning the DENV serotypes or genetic sequences of people who are infected with the virus, as well as the association between mild or severe illness either as a result of a primary infection or a secondary infection and the prevalent serotype in areas experiencing an epidemic, is necessary to forecast the epidemiological trends. Chikungunya outbreaks are notoriously difficult to forecast, both in terms of whether or not they will

happen and when they will. Although the development of new Chikungunya vaccines appears promising for controlling the disease, several hindrances must be overcome before they can be commercialised. The rapid proliferation of dengue, Chikungunya, and Zika viruses in recent times underscores the pressing necessity to discover scalable and economical methods for controlling Aedes aegypti. 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.

CONFLICT OF INTEREST / КОНФЛІКТ ІНТЕРЕСІВ

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS / ВКЛАД АВТОРІВ

All authors substantively contributed to the drafting of the initial and revised versions of this paper. They take full responsibility for the integrity of all aspects of the work.

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