



COINFECTION OF CHIKUNGUNYA AND DENGUE VIRUSES: A SEROLOGICAL STUDY FROM CENTRAL INDIA, INDIA

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ABSTRACT **INTRODUCTION:** Dengue and chikungunya (CHIK) infections appear to be increasing in all parts of India. *Aedes aegypti* mosquitoes are common vectors for dengue virus (DENV) and CHIK virus (CHIKV). In areas where both viruses cocirculate, they can be transmitted together. There are very few studies discussing the dengue-chik coinfection from Central region of India. The present study was undertaken to study the clinical features of dengue-CHIK coinfection and epidemiological factors. **MATERIALS AND METHODS:** IgM antibody capture (MAC) ELISA for dengue and Chikungunya, ELISA for (NS1 Ag) nonstructural protein 1 antigen odengue was performed on serum samples obtained from suspected patients. **RESULTS:** In this study, out of total 2287 suspected dengue fever cases, NS1 ELISA was positive in 283(12.37%) and IgM ELISA was positive in 506 (22.12%) cases, Total seropositivity of dengue was found in 789 (34.49%) cases. All positive 789 samples were subjected for IgMCHIK ELISA to rule out coinfection of them, 71 samples were found to be positive for Chikungunya. coinfection (dengue and chikungunya) was found in 8.99% of cases. males were affected more i.e 49 (69.01%) than females i.e 22 (30.98%), urban areas were found to be affected more with 47(66.19%) cases than rural area with 24 (33.80%) and coinfection was maximally seen in the age group of 21-30 years i.e 15 (21.12%) followed by 11-20 yrs i.e 13 (18.30%), coinfection was found more in the month of september 16 (22.53%), followed by in october 15(21.12%) and august. 14 (19.71%), All 71 (100%) coinfecting cases had a history of fever as a chief complaints followed by Arthralgia in 35(49.29%), 20 (28.16%) patients had a history of rash, 32 (45.07%) and 46 (64.78%) had thrombocytopenia and myalgia respectively. **CONCLUSION:** Increase in the number of Dengue and Chikungunya infections and their co-circulation is an important public health concern which warrants the implementation of strict control measures and timely diagnosis.

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

INTRODUCTION

Arthropod-borne viruses represent a global threat for public health as they can be transmitted to humans by bloodsucking vectors that are rapidly spreading worldwide. *Aedes aegypti* is an important vector mainly found in tropical and subtropical areas across the world and is implicated in the spread of several arboviruses; most important of them being dengue virus (DENV) and chikungunya virus (CHIKV). (10)

Dengue fever (DF) is a viral illness caused by 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 dandie" in Spanish. 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. In India, the first confirmed outbreak occurred in Kolkata in 1963-1964. Since then, there are many reports of dengue outbreaks from various parts of India. (10)

Chikungunya virus (CHIKV) was isolated in Tanganyika (now Tanzania) in 1953. In Asia, this virus is transmitted almost exclusively by *Aedes aegypti* mosquitoes. India had its first CHIKV outbreak in 1963; it was followed by epidemics in other parts of the country. Recently, massive outbreaks of CHIKV have been reported from many islands in the Indian Ocean. Chikungunya outbreaks in India were reported in 2005, and 1.4 million chikungunya cases were reported from different states. (16)

In Asia, the CHIKV-affected areas overlap with DENV-endemic areas 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. In India, concurrent isolation of CHIKV and DENV had been reported since 1964 from different states. (10)

MATERIALS AND METHODS:

The study was conducted in Virology Research and Diagnostic Laboratory, Indira Gandhi Government Medical College (I.G.G.M.C.), Nagpur during April 2019- December 2019. Blood samples were collected from patients attending different wards in hospital or visiting the outpatient departments at I.G.G.M.C and Hospital, Nagpur and

other districts nearby 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. NS1 Ag MICROELISA kits were provided by J. Mitra and Co. Pvt. Ltd. India. NIV DEN IgM Capture ELISA kit was provided by NIV, Pune, India, NIV CHIKUNGUNYA IgM Capture ELISA kit was provided by NIV, Pune, India. All the three tests were performed as per manufacturer's instructions. IgM antibody capture (MAC) ELISA for dengue and Chikungunya and ELISA for (NS1 Ag) nonstructural protein 1 antigen of dengue was performed on serum samples obtained from suspected patients.

RESULTS AND OBSERVATIONS:

Total 2287 samples received for dengue fever of them, 789 samples were found to be positive by NS1 ELISA & IgM ELISA having seropositivity of dengue in 34.49%. All positive 789 samples were subjected for IgMCHIK ELISA, of them, 71 samples were found to be positive for Chikungunya. Coinfection of dengue and chikungunya was found to be in 8.99% of fever cases.

Table 1:- Dengue test positivity by NS1 & IgM ELISA :

Sr.no	Tests	Total number of samples, n= 2287	
		No of positive cases	No. of negative cases
1.	NS1 ELISA	283(12.37%)	600 (26.24%)
2.	IgM ELISA	506 (22.12%)	898(39.27%)
3.	Total	789 (34.49%)	1498 (65.51%)

Table 1 shows, Total 2287 suspected fever cases were subjected for Dengue NS1 and IgM ELISA of them, NS1 ELISA alone was found to be positive in 283(12.37%) and IgM ELISA alone was positive in 506 (22.12%) cases. Total positivity was found to be 789 (34.49%) and 1498 (65.51%) were negative.

Table 2: Co-infection of dengue and chikungunya

Tests	Total samples tested	Total samples positive	Percentage positivity
Ns1 + IgM ELISA Dengue V	2287	789	34.49%
CHIK IgM MAC ELISA	789	71	8.99%
(CHIK + DEN) IgM MAC ELISA	789	71	8.99%

Dengue positivity

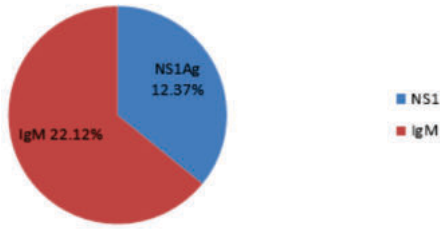


Table 2 shows, total 789 dengue positive cases were tested for Chikungunya by CHIK IgM ELISA of them, 71 (8.99%) were found to be positive ,having coinfection rate of 8.99% (71/789) by both viruses.

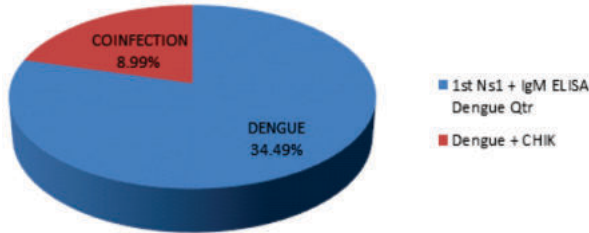


Table 3: Gender wise distribution of coinfectd cases:

Sr.no	Gender	No of coinfectd patients
1.	Male	49 (69.02%)
2.	Female	22 (30.98%)
3.	Total	71(100%)

Table 3 shows out of total 71 coinfectd cases, 49 (69.02%) were males and 22 (30.98%) were females

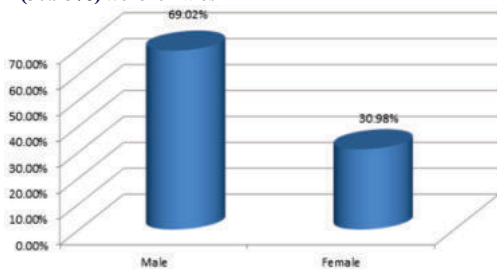


Table 4: Areawise distribution of coinfectd cases:

Sr.no	Area	No of coinfectd patients
1.	Urban	47(66.20%)
2.	Rural	24 (33.80%)
3.	Total	71(100%)

Table 4 shows out of total 71 coinfectd cases, 47(66.20%) coinfectd patients were from urban areas and 24 (33.80%) were from rural areas

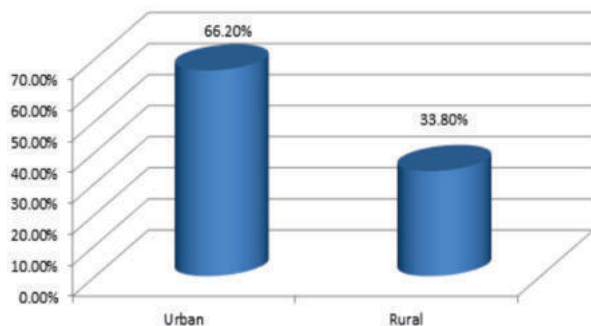


Table 5: Age wise distribution of coinfectd cases:

Sr.no	Age groups (yrs)	No of coinfectd
1.	0-10	10(14.08%)
2.	11-20	13 (18.30%)
3.	21-30	15 (21.13%)
4.	31-40	12 (16.90%)
5.	41-50	9 (12.68%)
6.	51-60	7 (9.87%)
7.	61-70	5 (7.04%)
8.	Total	71 (100%)

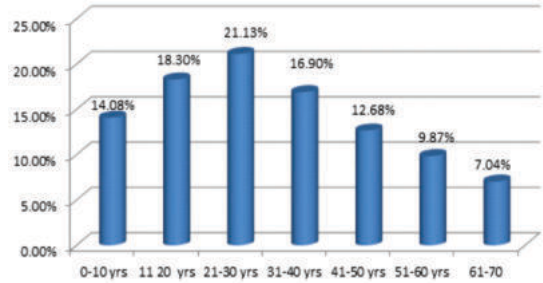


Table 5 shows out of total 71 coinfectd cases, maximum number of coinfection positivity of 15 (21.13%) was found to be in the age group of 21-30 years, followed by 13 (18.30%) in the age group of 11-20years, 12 (16.90%) were in 31-40 years, 10(14.08%) were seen in 0-10 years, 9 (12.68%) in 41-50 years and 5 (7.04%) cases were in 61-70 years.

Table 6: Month wise distribution of coinfectd cases

Sr.no	Months	No of coinfectd patients , n=71
1	April ,19	3 (4.22%)
2	May ,19	2 (2.82%)
3	June,19	4(5.63%)
4	July,19	8 (11.27%)
5	August,19	14 (19.72%)
6	September,19	16 (22.54%)
7	October,19	15(21.13%)
8	November,19	7 (9.86%)
9	December,19	2 (2.81%)
10	Total	71 (100%)



Table 6 shows ,coinfection of dengue and chikungunya was found to be more in the month of september 16 (22.53%), followed by 15 (21.12%) in october and 14 (19.71%) in august.

Table 7: Clinical manifeststions in coinfectd cases

Sr.no	Clinical signs & symptoms	No of coinfectd patients, (n=71)
1.	Fever	71(100%)
2.	Myalgia	46 (64.78%)
3.	Arthralgia	35(49.29%)
4.	Thrombocytopenia	32 (45.07%)
5.	Rash skin	20 (28.16%)

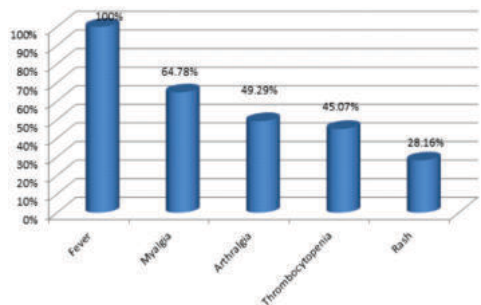


Table 7 shows, that All 71 (100%) coinfectd cases had a histroy of fever as a chief complaints followed by myalgia in 46 (64.78%), 35 (49.29%) patients had a histroy of arthralgia , 32 (45.07%) and 20 (28.16%) had thrombocytopenia and rash respectively.

DISCUSSION

The present study was conducted in a tertiary care hospital and a sentinel surveillance centre under NVBDCP. Study was carried out from april 2021 to December 2021 and total 2287 serum samples from suspected dengue fever cases were included.

Dengue and chikungunya fever are globally important arboviral infections. The first outbreak of the CHIKV in India occurred in 1963 in Kolkata followed by epidemics in Tamil Nadu, Andhra Pradesh, and Maharashtra. By 2010, the disease had spread to more than 18 states/union territories within the country. Species of mosquitoes, that is, *Aedes aegypti* as a principle vector and *Aedes albopictus* as a secondary vector. These infections have many common clinical features such as high-grade fever, rashes, nausea, headache, and body pain, so it is not always easy to differentiate the two infections clinically. Chikungunya fever is often misdiagnosed as dengue viral infection. In most cases of mild infection, symptoms subside spontaneously because the viral titer decreases in about 10 days.⁽⁴⁾

In the present study, out of 2287 suspected dengue fever cases, 789 cases were positive by IgM ELISA and NS1Ag. Seropositivity of dengue fever was found to be 34.49%. Our findings were similar to *Dr Kanizfatma Durani et al (2014)*⁽²⁾ who reported positivity of dengue in 34.5% in Jamnagar, Gujarat region while *Dhruba Hari Chandi (2020)*⁽³⁾ reported dengue seroprevalance 31.5% at Bhilai, Chhattisgarh region.

In the present study, out of 2287 suspected fever cases, 283 (12.37%) were positive for NS1 Antigen by ELISA and 600 (26.24%) cases were negative. Our findings are in accordance with *Hamzullah Khan (2021)*⁽⁴⁾ who observed 12% positivity for NS1 Antigen.

Out of 2287 suspected fever cases, 506 (22.12%) cases were found to be positive by IgM ELISA and 898 (39.27%) were negative. Our findings are in accordance with *Sandeep Kumar pal et al (2020)*⁽⁵⁾ reported IgM positivity in 22.22% cases of suspected dengue fever cases.

In the present study out of total 789 dengue positive cases of 71 (8.99%) were also positive for chikungunya showing co-infections of both.

Our findings are in accordance with the study done by *Rohit Sagar et al (2021)*⁽⁶⁾ and *Dr. Banwari Lal et al (2020)*⁽⁷⁾ who found co-infection in 10.34% and 8.60%. Study done by *M. Hisamuddin et al (2018)*⁽⁸⁾ and by *Vikram Londhey (2015)*⁽⁹⁾ reported Concurrent infection with both the viruses (Dengue and Chikungunya) was detected in 9% cases and 6.7% respectively. *Maninder Kaur et al (2018)*⁽¹⁰⁾ who found coinfection of dengue and chikungunya fever in 9.54% cases.

Out of total 71 coinfecting cases, males were affected more i.e 49 (69.01%) than females i.e 22 (30.98%). Our findings are similar with the study done by *Swati Gupta et al (2020)*⁽¹¹⁾ who also reported male preponderance of 66.6% in co-infected patients. Males are more involved in outdoor activities and hence, more exposed to the mosquito bites. Thus, male preponderance was seen. *Anju Dinkar et al (2017)*⁽¹²⁾ and *Dr. Banwari Lal et al (2020)*⁽⁷⁾ also found Males were predominance in their studies.

In the present study, the total 71 coinfecting cases, patients living in urban areas were found to be affected more with 47 (66.19%) cases than rural area with 24 (33.80%). Similar findings were reported by *Duane J. Gubler (2011)*⁽¹³⁾ who reported Unplanned urbanization and Globalization as one of the cause of increase dengue infection.

Maximum positivity of coinfection were seen in the age group of 21-30 years i.e 15 (21.12%) followed by 11-20 yrs i.e 13 (18.30%). *Dr. Banwari Lal et al (2020)*⁽⁷⁾ showed majority of the patients suffering from Dengue and Chikungunya infections belonged to the age group 21-40 years, followed by 11-20 yrs and very few cases below 10yrs and above 50yrs. *Shajji Dawood et al (2021)*⁽¹⁴⁾ also found the highest coinfection rate in the age group of 21-30yrs.

Coinfection of oth viral disease was found 16 (22.53%) more in the month of september, followed by 15 (21.12%) in october and in 14 (19.71%) august. Our findings are similar with study done by *Dr. Banwari Lal et al (2020)*⁽⁷⁾ who reported peaks from September to November and very few cases were in the other months.

Shajji Dawood et al (2021)⁽¹⁴⁾ found there was a gradual rise of cases during the post monsoon period. during the post monsoon season mosquitoes area abundantly present. The peak of both coinfection and monoinfection was observed between first week of October to mid-November every time.

All 71 (100%) coinfecting cases had a history of fever as a chief

complaints followed by myalgia in 46 (64.78%), 35 (49.29%) patients had a history of arthralgia, 32 (45.07%) and 20 (28.16%) had thrombocytopenia and rash respectively.

Study done by *Jitendra Singha et al (2017)*⁽¹⁵⁾ reported that Fever, Headache, Myalgia/body ache and Joint pain was found in all 100% coinfecting cases, Low backache in 46.6% and Nausea/vomiting in 23.1% cases while *Rohit Sagar et al (2021)*⁽⁶⁾ found Headache as a chief complaint in 75% cases followed by Joint pain in 75% cases, Vomiting in 50% and Rash in 25% cases. Similar findings were also observed by *Harendra S. Chahar et al (2009)*⁽¹⁵⁾ where all patients co-infected with dengue and chikungunya had fever, headache, joint pain, and low thrombocyte counts (<100,000/mm³).

Control:

Dengue and chikungunya has similar clinical presentations, even coinfection may result in illness with overlapping sign and symptoms, making diagnosis and treatment difficult for physicians. Thus, in clinically suspected cases of dengue and chikungunya fever, it is advisable to test for both viruses especially in areas where they co-exist. As there is no antiviral drug available, treatment is mainly based on supportive and nutritional care.⁽¹⁴⁾

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