SEROPREVALANCE OF DENGUE, CHIKUNGUNYA AND THEIR CO-INFECTION AT A TERTIARY CARE HOSPITAL.

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ABSTRACT

Introduction: Dengue and Chikungunya have re-emerged as important diseases of global concern. Both Dengue virus and Chikungunya virus are two rapidly spreading Arboviruses sharing common mode of transmission, i.e., through same species of mosquitoes. Therefore, these infections can be present in same geographical locations. Many clinical presentations are similar for both Dengue virus and Chikungunya virus. Their co-infection could have serious outcome, if not diagnosed and managed optimally.

Aim & Objectives: To know the seroprevalance of dengue, chikungunya, and their coinfections at tertiary care hospital.

Materials and methods: Blood samples of the patients presenting with fever, chills, headache, myalgia, arthralgia & vomiting were collected from April 2022 to September 2022 and ELISA test was performed to detect the presence of IgM antibodies against CHIKV and DENV.

Results: Out of 1612 samples tested, 324(20.09%) were found to be positive for DENV and 239(14.82%) for CHIKV antibodies. 69(4.28%) were found to be positive for both DENV and CHIKV. Majority of the positives are males 51(73.91%) and 18(26.08%) were females. 21-30yrs of age group has highest number of cases (44.92%) followed by 31-40yrs(31.88%). The predominant symptoms were fever in 99% and myalgia in 67% of patients.

Conclusions: Due to rapid urbanization, co-infection with DENV and CHIKV is becoming more prevalent in various areas of India. So enhanced surveillance to differentiate DENV and CHIKV infections clinically and diagnostically is needed for

early recognition of virus invasion and local transmission, better patient care and timely control measures.

Keywords: Dengue, Chikungunya, Co-infection, ELISA-Enzyme linked immunosorbent assay.

MAIN RESEARCH PAPER

INTRODUCTION:

Arthropod-borne viruses presents as worldwide global threat for public health. Aedes mosquito acts as the vector for transmission of both these infections. They bite during day time^{1,2}. It is an important vector mainly found in tropical and subtropical areas across the world³ and is implicated in the transmission of several arboviruses, most important being dengue virus (DENV) and chikungunya virus (CHIKV).

Dengue is caused by a single-strandedpositive-sense RNA virus known as dengue virus (DENV) belonging to the family Flaviviridae genus Flavivirus. The virus has four antigenically distinct serotypes (DENV-1 to 4)⁴, which are maintained in human-to-mosquito-to human transmission cycle.⁵In addition to these previously known four serotypes, a fifth serotype, DENV-5, circulating in the macaques has also been proposed from Malaysia in 2013.⁶

As Dengue mainly affects tropical and subtropical regions of the world, it is also prevalent throughout India. Majority of the cases have been reported from Kerala, Tamilnadu, Karnataka, Orissa, Delhi, Maharashtra and Gujarat.⁷ Symptoms of dengue may range from a mild febrile illness to severe illnesses like dengue fever (DF) and dengue hemorrhagic fever(DHF). When a person is infected with any one serotype for the first time, hewill develop a primary infection. Infection with one of the serotypes of DENV, will impart lifelong immunity against that particular serotype and temporary partial immunity against the other serotypes. Secondary infection with the different serotypes increases the risk of severe dengue hemorrhagic fever.⁸ Secondary dengue infection occurs in persons infected with second serotype which is different from first serotype and it will manifest as a severe form of illness such as DHF due to immune enhancement.^{9,10}

CHIK fever is a viral disease which is caused by an alpha virus that spreads by bite of Aedes aegypti mosquito. The name is derived from the Makonde word meaning that which bends up in reference to the stooped posture developed as a result of the arthritic symptoms of the disease.¹¹ CHIKV first established its presence during a 1952–1953 epidemic outbreak in Tanzania.¹² In India, CHIKV was first isolated in

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Calcutta in 1963. The virus disappeared from our country after last reports from Maharashtra in 1973.¹³ It then reemerged in 2006 after a gap of 32 years and caused an explosive outbreak affecting 13 states.¹⁴

Clinical presentations of these two infections are also similar, especially in initial stages characterized by fever, rash, myalgia and Arthralgia¹⁵. In many hospitals, as mortality rate and severity is high in dengue fever as compared to chikungunya, most of the patients are tested only for dengue virus and in only rare cases they undergo tests for chikungunya infection. As a result, burden of chikungunya has been missed and also many cases go undiagnosed.¹⁶ The environment and man made factors like increased urbanization, poor sanitation, overcrowding, man made breeding sites and climatic changes favours the infection and are responsible for the spread of the disease. High temperature and high humidity prolongs the life span and spread of the vector.^{17,18,19}

Based on clinical presentation, diagnosis& differentiation of dengue and chikungunya is difficult. Although, majority of the infections are self-limiting, timely diagnosis of dengue helps in appropriate management in severe cases.²⁰ Enzyme-linked immunosorbent assays (ELISAs), RT-PCR and virus isolation tests helps in diagnosis of these infections. ELISA can detect both immunoglobulins M (IgM) and G (IgG) from samples. Detection of dengue non-structural antigen (NS1 Ag) may help in the early diagnosis and treatment of dengue.^{21,22}Early diagnosis is essential for the early and appropriate treatment and for implementation of control measures. The present study is conducted to know the seroprevalance of dengue, chikungunya and their co infection at a tertiary care hospital.

Materials and methods

This prospective study was done at Microbiology department of a Tertiary care hospital in South India for duration of 6 months from April 2022 to September 2022.

4-5 ml of blood from the patients with suspected signs & symptoms of dengue and chikungunya were collected at both outpatients and inpatients. All the collected samples were centrifuged and sera was separated and ELISA test was performed to detect presence of Immunoglobulin M(IgM) antibodies against CHIKV and DENV by IgM antibody capture MAC ELISA kits (Arbovirus diagnostics NIV, Pune,India)at our VRDL(viral research and diagnostic laboratory), Tertiary care hospital. All the tests were performed by strictly following kit manufacturer's instructions.

Results

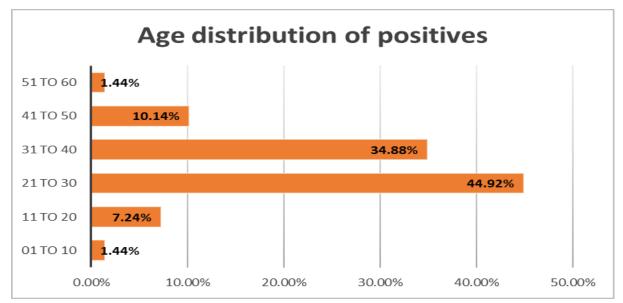
A total of 1612 blood samples were received for dengue and chikungunya IgM antibody capture ELISA testing, 324(20.09%) were found to be positive for DENV IgM, out of which 223(68.82%) were males and 101(31.17%) were females. 239(14.82%) were found positive for CHIKV among which 141(58.99%) were males and 98(41%) were females. Co-infection with DENV and CHIKV was found in 69(4.28%) (Table 1). Majority of the co-infected patients were males 51(73.91%) and 18 (26.08%) were females(Figure 2). The common age group affected was 21-31yrs (44.92%) followed by 31-40yrs(31.88%)(Figure 1).

The predominant symptoms seen among these patients were fever (99%) and myalgia (67%) followed by headache (34%), arthralgia (29%), rashes (27%) and vomiting (13%)(Table 2).

S.No	Infection	Positives(n=1612)	Percentage (%)
1.	DengueIgM	324	20.09%
2.	ChikungunyaIgM	239	14.82%
3.	Co-infection	69	4.28%

Table 1 SHOWING RATE OF POSITIVES

FIGURE 1 SHOWING AGE WISE DISTRIBUTION OF PATIENTS



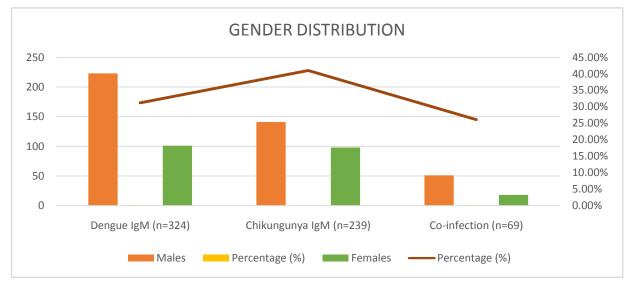


Figure 2 SHOWING GENDER DISTRIBUTION OF POSITIVES

Table 2 SHOWING CLINICAL MANIFESTATIONS OF PATIENTS

S.NO	Signs and symptoms (n=1612)	Percentage %
1.	Fever	99%
2.	Myalgia	67%
3.	Headache	34%
4.	Arthralgia	29%
5.	Rashes	27%
6.	Vomiting	13%

Discussion

Dengue virus belongs to genus Flavivirus and chikungunya to an Alpha virus.¹⁴ These Arboviral infections, transmitted by Aedes Aegypti mosquito are of great concern for public health. Both these viruses may co-circulate and can be transmitted together.²³ Changes in the genotype and mutations in the genome have been detected for both dengue and chikungunya viruses¹⁴. Appropriate management of patient requires accurate and early diagnosis of infection.

In present study, seroprevalance of dengue IgM antibody was 20.09% similar to study done by Shah PS et al. who reported $20.05\%^{24}$ and higher than the study of Nepal H.P et al. Which showed 8.5%.²⁵

Seroprevalance of chikungunya IgM antibody was 14.82%, correlating with the study done by Ms.Akanksha Tomar et al. which showed seroprevalence of 16%.²⁶

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In our study, males were commonly affected with DENV (68.82%) which is higher than the study done by R. Ganesan et al. who reported it as 51.9%.²⁷ Males (58.99%) were more commonly affected with CHIKV than females (41%), in contrast to the study conducted by Cueva J.T.D et al. which showed 68.9% females out of the 777 confirmed cases of chikungunya.²⁸ Gender differences are common due to community-specific habits, customs or behaviors.²⁹

Co infection with DENV and CHIKV was seen in 4.28% patients with male (73.91%) predominance over females (26.08%). The study done by Gupta S..et al reported similar seroprevalance as $4.5\%^{30}$ and another study by Kaur M.,et al showed 9.54%.³¹

Serological tests are the most used diagnostic methods. But these tests cannot identify the serotype causing infection. Polymerase chain reaction (PCR) is becoming the rapid detection method and can be used for detection of serotypes and quantification of viral load.³² Diagnosis of dengue and chikungunya becomes difficult without adequate serological and other diagnostic tests. More studies are required to know the prevalence of these infections and their co infection in particular areas, to prevent transmission of the disease and implement effective control measures.

Conclusion

The incidence for co-infection of DENV and CHIKV is increasing due to rapid urbanization, feeding behavior of mosquitoes, low socioeconomic conditions and increased international travel. Our study suggested that two viruses can co-exist in same host and serological investigations should be carried out for both the viruses in individuals showing sign of infection with either Dengue or Chikungunya. Therefore, enhanced surveillance is needed for early recognition of virus invasion and local transmission, better patient care and timely control measures by community awareness and vector control.

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Ethical approval: Ethical approval was taken from ethical committee of Kakatiya medical college, Hanumakonda.

REFERENCES:

Sudhan SS, Sharma M, Sharma P, Gupta RK, Sambyal SS, Sharma S. Serosurveillance of Dengue, Chikungunya and Zika in Jammu, a Sub-Himalayan Region of India. *J Clin Diagn Res.* 2017;11(11): DC05-DC08.doi: 10.7860/ JCDR/2017/29210.10848

- Kalawat U, Sharma KK, Reddy SG. Prevalence of dengue and chickungunya fever and their coinfection. Indian J Pathol Microbiol. 2011; 54(4): 844-846. doi: 10.4103/0377-4929.91518
- 3) Capinha C, Rocha J, Sousa CA. Macroclimate determines the global range limit of *Aedes aegypti*. Ecohealth 2014;11:420-8.
- 4) Gubler DJ (2002) Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. Trends in Microbiology 10: 100–103. https://doi.org/10.1016/s0966-842x (01)02288-0 PMID: 11827812
- 5) WHO (2018) Dengue vaccine: WHO position paper–September 2018. Geneva: World Health Organization. 457–476 p.
- 6) Normile D (2013) Surprising new dengue virus throws a spanner in disease control efforts. Science 342: 415–415. https://doi.org/10.1126/science.342.6157.415 PMID: 24159024
- 7) Katzelnick LC, Gresh L, Halloran ME, Mercado JC, Kuan G, et al. (2017) Antibody-dependent enhancement of severe dengue disease in humans. Science 358: 929–932. https://doi.org/10.1126/science.aan6836 PMID: 29097492
- 8) Modi KP, Patel DA, Vegad MM, Mistry AU, Padariay NJ, Rathod AB. Sero-Prevalence of Dengue and Chikungunya, their Co-Infection and Seasonal Trends of These Infections at a Tertiary Care Hospital, Ahmedabad, Gujarat. *Int J Microbiol Res.* 2017;9(1):819-822.
- Nguyen THT, Clapham HE, Phung KL, et al. Methods to discriminate primary fromsecondary dengue during acutesymptomatic infection. *BMC Infectious Diseases*. 2018;18:375. doi: 10.1186/s12879-018-3274-7
- 10) Khurram M, Qayyum W, Hassan SJ, Mumtaz S, Bushra HT, Umar M. Dengue hemorrhagic fever: Comparison of patients with primary and secondary infections. *Journal of Infection and Public Health.* 2014;7(6):489- 495. doi: 10.1016/j.jiph.2014.05.005
- 11) Chhabra M, Mittal V, Bhattacharya D, Rana U, Lal S. Chikungunya fever: A re-emerging viral infection. Indian J Med Microbiol 2008;26:5-12. 12
- 12) Khan AH, Morita K, Parquet Md Mdel C, Hasebe F, Mathenge EG, Igarashi A, et al. Complete nucleotide sequence of chikungunya virus and evidence for an internal polyadenylation site. J Gen Virol 2002;83:3075-84.
- 13) Chattopadhyay S, Mukherjee R, Nandi A, Bhattacharya N. Chikungunya virus infection in West Bengal, India. Indian J Med Microbiol 2016;34:213-5.
- 14) Cecilia D. Current status of dengue and Chikungunya in India. WHO South East Asia J Public Health 2014;3:22-6.
- 15) Shrihari N, Kumudini TS, Mariraj J, Krishna S. The Prevalence of Arboviral diseases mainly Dengue, Chikungunya and Japanese B Encephalitis in and around Bellary district, Karnataka. J Pharm Biomed Sci. 2012;15(10):1-3.
- 16) Dinkar A, Singh J, Prakash P, Das A, Nath G. Hidden burden of chikungunya in North India; A prospective study in a tertiary care centre. J Infect Public Health. 2017;11(4):586-591. doi: 10.1016/j.jiph.2017.09.008
- 17) Bhagwati C, M M, Mehta KD, Y S G. Profile of The Chikungunya Infection: A Neglected Vector Borne Disease which is Prevalent In The Rajkot District. J Clin Diagn Res. 2013;7(6):1008-1011. doi:10.7860/ JCDR/2013/5307.3057
- Selvakumari S. A study on detection of prevalence of dengue, chikungunya, leptospirosis and their coinfection in acute febrile patients. University Journal of Pre and Para Clinical Sciences. 2018;4(1).
- 19) Chandran R, Azeez PA. Outbreak of dengue in Tamil Nadu, India. Current Science. 2015;109(1):171-176.
- 20) Kajeguka DC, Kaaya RD, Mwakalinga S, et al. Prevalence of dengue and chikungunyavirus infections in northeastern Tanzania: a cross sectional study among participants presenting with malaria-like symptoms. BMC Infectious Diseases. 2016;16:183. doi: 10.1186/s12879-016-1511-5
- 21) Lertanekawattana S, Anantapreecha S, Jiraphongsa C, et al. Prevalence and characteristics of dengue and chikungunya infections among acute febrile patients in NongKhaiProvince, Thailand. Southeast Asian J Trop Med Public Health. 2013;44(5):780-790.
- 22) Deeba F, Afreen N, Islam A, et al. Co-infection with Dengue and Chikungunya Viruses. Current Topics in Chikungunya. 2016. doi: 10.5772/64308.
- 23) Giriraja KV, Pavitra C, Bindumathi PL. Seroprevalence of chikungunya and dengue dual infection and chikungunyamonoinfection among patients with acute febrile illness attending a medical research institute in Bangalore. J Evid Based Med Health. 2017; 4(25):1482- 85. doi: 10.18410/jebmh/2017/287
- 24) Shah PS, Alagarasu K, Karad S, Deoshatwar A, Jadhav SM, Raut T, Singh A, Dayaraj C, Padbidri VS. Seroprevalence and incidence of primary dengue infections among children in a rural region of Maharashtra, Western India. BMC Infectious Diseases. 2019 Dec;19(1):1-6.
- 25) Nepal HP, Ansari S, Gyawali N. Detection of IgM against Dengue Virus in Clinically Suspected Patients Presenting at a Tertiary Care Centre, Narayani Zone, Nepal. J Trop Dis. 2014; 2(3). doi: 10.4172/2329-891X.1000139
- 26) A Tomar, AVB Hodiwala, DD Khiste. Prevalence of Chikungunya Viral Infection in a Tertiary Care Hospital, Navi Mumbai Maharashtra. Journal of Medical Science and Clinical Research. 2017;5(1):15948-15951. doi: 10.18535/jmscr/v5i1.115
- 27) Ganesan R, Devamani TSD, Innocent DJP. A Study on the Prevalence of Dengue Virus Infection using NS1 Antigen and IgM Antibody capture ELISA for the Early Diagnosis in and around

Madurantakam, India. Int J Curr Microbiol App Sci. 2019;8(02):1596-1600. doi: 10.20546/ijcmas.2019.802.187

- Cueva JTD, Ples MB, Vitor RJS. Relationship between chikungunya virus prevalence, rainfall, and urbanization in the Philippines. Natl J Physiol Pharm Pharmacol. 2018;8:977-982. doi: 10.5455/ njppp.2018.8.0208204032018
- 29) NAM Azami, SA Salleh, SA Shah, et al. Emergence of chikungunya sero positivity in healthy Malaysian adults residing in outbreak-free locations: Chikungunya seroprevalence results from the Malaysian Cohort. BMC Infectious Diseases. 2013;13:67. doi: 10.1186/1471-2334-13-67.
- 30) GUPTA S, AGRAWAL S, SHASTRI J. Dengue and Chikungunya Mono and Co-infections among Patients with Acute Febrile Illness. Journal of Clinical & Diagnostic Research. 2020 Oct 1;14(10)
- 31) Kaur M, Singh K, Sidhu SK, Devi P, Kaur M, Soneja S, Singh N. Coinfection of chikungunya and dengue viruses: A serological study from North Western region of Punjab, India. Journal of laboratory physicians. 2018 Oct;10(04):443-7.
- 32) SOD PaulaI, BALd FonsecaI. Dengue: A Review of the Laboratory Testsa Clinician Must Know to Achieve a Correct Diagnosis. Braz J Infect Dis. 2004;8(6):390-398. doi: 10.1590/S1413-86702004000600002