Manish Kumar et al /J. Pharm. Sci. & Res. Vol. 15(8), 2023, 1194-1197

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Sero-epidemiological evaluation of dengue-chikungunya coinfections: A study from capital city of Uttarakhand

Manish Kumar[1],Minakshi Singh[1], Shweta Singhal[1],Nidhi Negi[1],Yogita Rawat[1], Shweta Thaledi[2], *Shekhar Pal[1,2]

[1]Department of Microbiology, [2]Viral Research & Diagnostic Laboratory, Government Doon Medical College, Patel Nagar, Dehradun, Uttarakhand

* drshekharpal@gmail.com

Abstract

Introduction: Arboviruses transmitted to humans via blood sucking vectors pose a global public health threat. Dengue and chikungunya caused by dengue virus (DENV) and chikungunya virus (CHKV) respectively are among the two most important and prevalent mosquito borne arboviral diseases across the world.

Aim: To know the sero-epidemiology of DENV and CHKV monoinfection as well as their co-infection in and around district Dehradun during an outbreak in 2022.

Materials and methods: Blood samples were collected from 1285 patients, clinically suspected of DENV and/or CHKV and were subjected to dengue NS1 antigen capture ELISA (fever <5 days); anti dengue IgM antibody capture ELISA (fever >5 days); both, dengue NS1 antigen and IgM antibody ELISA (fever 3-5 days). As per the request, samples were also tested for anti-CHKV IgM antibody capture ELISA. If requested, some samples were tested for both CHKV and DENV infection. The tests were carried out and results interpreted as per the manufacturer's instructions.

Results: Out of total of 1285 samples, 751 (58.44%) tested positive for DENV. Out of 534 samples tested for CHKV, 117 (21.9%) tested positive. 261 samples were tested for both DENV and CHKV out of which 43 (16.5%) samples were positive for dual infection. Majority of the patients belonged to age group of 21–40 years. Among the DENV mono-infected patients majority were males (57.7%) compared to CHKV mono-infected patients (54.7). Female preponderance (55.8%) was noted among patients with dual infection.

Conclusion: Co-infections with DENV/CHKV cause overlapping signs and symptoms and can pose patient-management as well as therapeutic challenges for clinicians. Thus both DENV and CHKV be tested simultaneously among patients suspected of DF or CF particularly in areas where both the viruses co-circulate.

Keywords: *Aedes aegypti,* Arboviruses, Arthralgia, Dengue hemorrhagic fever, Dengue shock syndrome, Expanded dengue syndrome

INTRODUCTION

Arboviruses transmitted to humans via blood sucking vectors pose a global public health threat and are primarily prevalent in tropical and subtropical areas across the world.[1] Dengue and chikungunya are among the two most important arboviral diseases of international concern to human health.[2]

Dengue fever (DF) is caused by a flavivirus (family Flaviviridae) known as dengue virus, which spreads mainly by the bite of female *Aedes aegypti* mosquito and to a lesser extent by *Ae.albopictus*.[3] Spectrum of disease ranges from self limiting DF to more severe and life threatening conditions like dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS).[4] The virus has four antigenically distinct serotypes (DENV-1 to 4).[5] Infection with one of the serotypes confers life long immunity from subsequent exposure to the same serotype and temporary partial imuunity against other serotypes.[6] Sequential infections from other serotypes increases the risk of DHF and/or DSS.[7] The global incidence of dengue is estimated to be 390 million cases per year of which 96 million manifest apparently.[8] In india the first confirmed dengue outbreak was reported from Kolkata in 1963-64 [9] and since than many outbreaks have been reported from various parts of the country.[10-12] Over the past decade a significant upsurge has been noted in the incidence of dengue infection from India, and as per the reports from National Vector Borne Disease Control Program (NVBDCP) >67,000 dengue cases were reported in year 2019.[8]

Chikungunya fever (CF) on the other hand is caused by an alphavirus (family Togaviridae) known as chikungunya virus (CHKV), and like DENV is also transmitted by the bite of *Ae.aegypti* mosquito.[13] Three distinct evolutionary clades of CHKV are known, namely: West African, Central/East African and Asian CHKV.[3] CHKV infection causes an acute illness with fever, rash and severe arthralgia which is also one of the important clinical symptoms. The joint pain is often incapacitating and usually lasts for few days, but can be prolonged lasting weeks, months and even years.[14] CHKV was first reported from an epidemic outbreak in Tanzania in year 1952-53,[15] and in India was reported for the first time from Kolkata in year 1963.[16] After the last reports from Maharashtra in year 1973, CHKV was considered to have been disappeared from India, however, after a gap of 32 years it re-emerged in 2006 and caused an explosive outbreak.[17]

CHKV and DENV share the common vector, symptoms and geographical distribution,[18] and in Asia, the CHKV affected areas overlap with DENV endemic areas, and provide opportunities for mosquitoes to become infected and transmit both the viruses simultaneously.[19] Co-

infection with DENV and CHKV has been reported from various states of India since 1964.[19-23]

According to NVBDCP, dengue is an endemic disease in Uttarakhand, with approximately 15,000 cases reported in the last six years. But for chikungunya infection, which otherwise is endemic in most parts of the country, only 163 confirmed cases have been reported from Uttarakhand in the last six years.[24] Even though, few cases have been reported, but considering the common vector, symptoms, geographical distribution and outbreak potential of both DENV and CHKV, the presence of CHKV in Uttarakhand cannot be ignored. With the above background and the paucity of data regarding the DENV and CHIKV co-infection from this region, this study was conducted to know the seroprevalence of DENV and CHKV monoinfection as well as their co-infection in and around district Dehradun during an outbreak in 2022.

MATERIALS AND METHODS

Acute phase blood samples were collected from 1285 patients, clinically suspected of DENV and/or CHKV and were sent for investigations to Medical College level Virology Research and Diagnostic Laboratory (VRDL) in the Dept. of Microbiology, Govt. Doon Medical College (GDMC), Dehradun. Approximately, 2–5 ml of blood was collected, serum separated, and subjected to ELISA based tests. As the samples were received during the outbreak (June 2022 to December 2022), ethical clearance was not required.

Patients with history of fever for <5 days were tested for dengue NS1 antigen by capture ELISA (Panbio Dengue Early ELISA, Panbio Diagnostics, Brisbane, Australia) while patients having fever for >5 days were tested for dengue specific IgM antibody by IgM antibody capture (MAC) ELISA, provided by National Institute of Virology (NIV), Pune. Patients having a history of 3–5 days of fever were tested for both dengue NS1 antigen and IgM antibodies in the serum. Using MAC-ELISA kit provided by NIV, Pune, IgM antibodies against CHKV were detected in the samples with request for testing CHKV infection. As per the requisition some of the samples were tested for both CHKV and DENV infection. All the tests were carried out and results interpreted as per the manufacturer's instructions.

Although due to the limited resources, simultaneous molecular confirmation of the ELISA results could not be done, but the samples tested positive were stored at -80°C for any further molecular investigation, serotyping or sequencing if planned in future.

RESULTS

Out of total of 1285 samples suspected of DENV and/or CHKV, 751 (58.44%) tested positive for DENV of which 419 (74.3%) samples were positive for NS1 antigen, 182 (32.3%) were positive for dengue IgM antibody and 37 (6.6%) samples were positive for both NS1 antigen as well as IgM antibody. All the samples found negative for DENV (*n*=534) were tested for anti CHKV IgM antibodies out of which 117 (21.9%) tested positive. Requisition for testing both DENV and CHKV was received for 261 samples out of which 43 (16.5%) samples were found positive for dual infection. Age wise distribution of the DENV and CHKV infected patients revealed that majority of the patients belonged to age group of 21–40 years. Among the DENV mono-infected patients majority were males (57.7%) compared to CHKV mono-infected patients, where females (54.7%) predominated males. Among the patients with co-infection also female preponderance (55.8%) was noted. The seasonal distribution of dengue cases showed a peak during the month of August while maximum number of chikungunya cases, and co-infection with DENV and CHKV were seen during the month of September. Table 1 depicts the age, gender and month wise distribution of the DENV and/or CHKV mono-infection and/or co-infection.

Table 1: Age, gender and month wise distribution of the DENV and/or CHKV mono-infection and/or co-infection.

Distribution	Dengue positive (%) *n*=751	Chikungunya positive (%) *n*=117	Co-infection (%) *n*=43
Age wise distribution (in years)			
0-20 `	148 (19.7)	19 (16.2)	2 (4.7)
21-40	312 (41.5)	45 (38.5)	19 (44.2)
41-60	193 (25.7)	31 (26.5)	13 (30.2)
61-80	77 (10.3)	13 (11.1)	5 (11.6)
>80	21 (2.8)	09 (7.7)	4 (9.3)
Gender Distribution			
Male	433 (57.7)	53 (45.3)	19 (44.2)
Female	318 (42.3)	64 (54.7)	24 (55.8)
Month wise distribution			
June	36 (4.8)	07 (6.0)	02 (4.7)
July	67 (8.9)	15 (12.8)	07 (16.3)
August	194 (25.8)	29 (24.8)	08 (18.6)
September	168 (22.4)	37 (31.6)	13 (30.2)
October	155 (20.6)	16 (13.7)	05 (11.6)
November	83 (11.1)	10 (8.5)	04 (9.3)
December	48 (6.4)	03 (2.6)	04 (9.3)

DISCUSSION

DENV infections are endemic in Northern India and in recent years, increasing trends of the co-circulation of multiple DENV serotypes suggest that DENVs are becoming hyperendemic. Rapid expansion in the geographical extent of DENV is being increasingly observed all over the country including Uttarakhand state where recently in 2018-19 a major outbreak of dengue was also witnessed. Multiple factors are responsible for such expansion, primarily being, deforestation/urbanisation/globalisation resulting in climatic and environmental changes providing increased opportunity for pathogen and vector to spread. [3] In the current study the incidence of DENV was found to be 58.44% and maximum number of cases were noted from August to October, which is a post monsoon period. The climatic conditions during this period favour vector breeding places, thereby increasing the vector population and resulting in rise of such vector borne diseases.

The World Health Organization, in 2012, coined the term "expanded dengue syndrome" (EDS) to describe patients that do not fit into either DHF or DSS but show atypical symptoms in vital organ systems such as the cardiovascular, neurological, renal, gastro intestinal, and hematological system.[25,26] EDS is usually associated with dengue co-infection with other viral, bacterial, immunological and parasitic diseases. Various studies from India have shown DENV and CHKV co-infection to be the most common.[4,27] In the present study the incidence of DENV-CHKV co-infection was found to be 16.5% (*n*=261), which is similar to the incidence reported in previous Indian studies as well.[4,27] Maximum number of cases were recorded among the age group of 21-40 years, a finding which is in tandem with the previous report by Lall *et al*. from Delhi.[28] We speculate that being occupationally active and involved in outdoor activities, this age group has higher probability of acquiring such infections.

In order to formulate/tailor the prevention and treatment strategies, it is imperative to know the epidemiology, transmission dynamics and the burden of disease in a given area. However, as the clinical features of DENV and CHKV are similar, CHKV infections may often go undiagnosed in DENV endemic areas. Moreover in India, *Ae.aegypti* mosquitoes are the primary vectors for both DENV and CHKV thus the co-infection might be carried out by consecutive bites of two female mosquitoes each carrying a different virus or by the bite of a single female mosquito co-infected with both the viruses.[6] Opportunities for DENV-CHKV co-infections to occur in humans are increased by the feeding behaviour of the mosquito, low socio-economic conditions and high population density.[19] CHKV although prevalent in almost every part of the country,[29] very few cases have been reported from Uttarakhnad. We believe that, this under reporting/under diagnosis of CHKV can be due to, CHKV being ignored in the presence of DENV infection. As per NVBDCP, DENV is endemic in Uttarakhand and as both DENV and CHKV present with similar clinical manifestation and haematological laboratory investigation, there is high probability that once the infection with DENV is ascertained (laboratory confirmed), screening for CHKV is not done or is ignored. Moreover, due to lack of information about the actual burden of CHKV in this region, the clinicians sometimes tend to ignore testing for CHKV, even in the dengue negative (but symptomatic) cases which further contributes to the under diagnosis/under reporting of CHKV infection.

Our study results corroborate well with the previous reports by various other researchers that DENV/CHKV co-infections occur in areas where these two viruses co-circulate. Repeated outbreaks of dengue, recent upsurge in the CHKV cases and DENV/CHKV co-infections indicate that these viruses are becoming hyperendemic in Uttarakhand as well. Simultaneous infections with both the viruses cause overlapping signs and symptoms and can pose patient-management as well as therapeutic challenges for clinicians. Thus in clinically suspected cases of DF or CF, testing for both DENV and CHKV is recommended, particularly in areas where they co-circulate.

CONCLUSION

We report co-infections with DENV and CHKV from district Dehradun Uttarakhand. As of now it is difficult to comment on increased disease severity in patients with DENV/CHKV co-infections, as additional clinical information is required to determine the same. Future studies conducted in this regard will surely be helpful to determine the influence of co-infections on disease severity and associated complications if any. Such studies will also be useful in monitoring the spread of these arboviruses and implementing appropriate control strategies. Of note, both DENV and CHKV be tested simultaneously in patients suspected of DF or CF.

REFERENCES

1. Capinha C, Rocha J, Sousa CA. Macroclimate determines the global range limit of Aedes aegypti. Ecohealth 2014;11:420-8
2. Badoni G, Gupta PK, Gupta P, Kaistha N, Mathuria YP, Pai MO, et al. Dengue-chikungunya infection in the tertiary care hospital of northern India: Cross-sectional latent class cluster analysis in viral infection. Heliyon. 2023;9(3):e14019
3. Furuya-Kanamori L, Liang S, Milinovich G, Soares Magalhaes RJ, Clements AC, Hu W, et al. Co-distribution and co-infection of chikungunya and dengue viruses. BMC Infect Dis. 2016;16:84
4. Kaur M, Singh K, Sidhu SK, Devi P, Kaur M, Soneja S, et al. Coinfection of chikungunya and dengue viruses: A serological study from North Western region of Punjab, India. J Lab Physicians. 2018;10(4):443-447
5. Dengue vaccine: WHO position paper, September 2018 - Recommendations. Vaccine. 2019 Aug 14;37(35):4848-4849
6. Raza FA, Javed H, Khan MM, Ullah O, Fatima A, Zaheer M, et al. Dengue and Chikungunya virus co-infection in major metropolitan cities of provinces of Punjab and Khyber Pakhtunkhwa: A multi-center study. PLoS Negl Trop Dis. 2021;15(9):e0009802
7. Katzelnick LC, Gresh L, Halloran ME, Mercado JC, Kuan G, Gordon A, et al. Antibody-dependent enhancement of severe dengue disease in humans. Science. 2017;358(6365):929-932
8. Turuk J, Palo SK, Rath S, Subhadra S, Sabat J, Sahoo PK, et al. Viral characteristics and clinical presentation in dengue co-infection- Findings from a facility based observational study in Odisha, India. J Family Med Prim Care 2021;10:2958-63

9. Sarkar JK, Chatterjee SN, Chakravarty SK. Haemorrhagic fever in Calcutta: Some epidemiological observations. Indian J Med Res 1964;52:651-9
10. Vikram K, Nagpal BN, Pande V, Srivastava A, Saxena R, Anvikar A, et al. An epidemiological study of dengue in Delhi, India. Acta Trop. 2016;153:21-7
11. Mutheneni SR, Morse AP, Caminade C, Upadhyayula SM. Dengue burden in India: recent trends and importance of climatic parameters. Emerg Microbes Infect. 2017;6(8):e70
12. NVBDCP. DENGUE/DHF situation in India: national center for vector borne diseases control (NCVBDC), 2022. Available from: https://nvbdcp.gov.in/index4.php?lang=1&level=0&linkid=431&lid=3715, 2022 [Last accessed on February 13, 2023]
13. Fernández-Salas I, Díaz-González EE, López-Gatell H, Alpuche-Aranda C. Chikugunya and zika virus dissemination in the Americas: different arboviruses reflecting the same spreading routes and poor vector-control policies. Curr Opin Infect Dis. 2016;29(5):467-75
14. World Health Organization. Chikungunya. WHO, 2022. Available from: https://www.who.int/health-topics/chikungunya#tab=tab_1 [Last accessed on February 25, 2023]
15. 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
16. Chattopadhyay S, Mukherjee R, Nandi A, Bhattacharya N. Chikungunya virus infection in West Bengal, India. Indian J Med Microbiol 2016;34:213-5
17. Ravi V. Re-emergence of chikungunya virus in India. Indian J Med Microbiol 2006;24:83–4
18. Weaver SC, Charlier C, Vasilakis N, Lecuit M. Zika, Chikungunya, and Other Emerging Vector-Borne Viral Diseases. Annu Rev Med. 2018;69:395-408
19. Chahar HS, Bharaj P, Dar L, Guleria R, Kabra SK, Broor S. Co-infections with chikungunya virus and dengue virus in Delhi, India. Emerg Infect Dis. 2009;15(7):1077-80
20. Yergolkar PN, Tandale BV, Arankalle VA, Sathe PS, Sudeep AB, Gandhe SS, et al. Chikungunya outbreaks caused by African genotype, India. Emerg Infect Dis. 2006;12:1580–3
21. Nayar SK, Noridah O, Paranthaman V, Ranjit K, Norizah I, Chem YK, et al. Co-infection of dengue virus and chikungunya virus in two patients with acute febrile illness. Med J Malaysia 2007;62:335-6
22. Kalawat U, Sharma KK, Reddy SG. Prevalence of dengue and chickungunya fever and their co-infection. Indian J Pathol Microbiol 2011;54:844-6
23. Taraphdar D, Sarkar A, Mukhopadhyay BB, Chatterjee S. A comparative study of clinical features between monotypic and dual infection cases with chikungunya virus and dengue virus in West Bengal, India. Am J Trop Med Hyg 2012;86:720-3
24. NVBDCP. Chikungunya situation in India. national center for vector borne diseases control (NCVBDC). Available from: https://nvbdcp.gov.in/index4.php?lang=1&level=0&linkid=486&lid=3765, 2022 [Last accessed on March 11, 2023]
25. Umakanth M, Suganthan N. Unusual manifestations of dengue fever: a review on expanded dengue syndrome. *Cureus* 2020;12:e10678
26. Mohanty B, Sunder A, Pathak S. Clinicolaboratory profile of expanded dengue syndrome – Our experience in a teaching hospital. J Fam Med Prim Care 2019;8:1022-7
27. Singh J, Dinkar A, Singh RG, Siddiqui MS, Sinha N, Singh SK. Clinical profile of dengue fever and coinfection with chikungunya. Ci Ji Yi Xue Za Zhi. 2018;30(3):158-164
28. Lall H, Gupta P, Debbarma M, Sharma P, Ansari SK, Jais M, et al. Seroprevalence of dengue in tertiary care hospital in Delhi. International Journal of Current Microbiol and Applied Sciences 2016; 5:439-45
29. Translational Research Consortia (TRC) for Chikungunya Virus in India. Current Status of Chikungunya in India. Front Microbiol. 2021 Jun 24;12:695173