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Research Article

EPIDEMIOLOGY AND CLINICAL PROFILE OF DENGUE AND CHIKUNGUNYA INFECTION AND SEROTYPE DIVERSITY OF DENGUE VIRUS IN NORTHERN INDIA

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ABSTRACT

Objectives: Dengue and chikungunya (CHIKV) are major public health problem and are notable arthropod viral infections due to their expanding geographical distribution. As there are multiple serotypes of dengue virus circulating concurrently with CHIKV virus, the present study was conducted to describe the seroprevalence, epidemiological characteristics, clinical profile, seasonal trends, coinfection, and prevalent circulating dengue virus serotypes (DENVs).

Methods: Serum samples from clinically suspected cases of dengue and CHIKV were subjected to serological testing by performing enzyme-linked immunosorbent assay. The particulars of the patient including case history, demographic characteristics, co-morbidities, clinical features, and evolution of symptoms were recorded. Further, samples which were positive for NS1 antigen were subjected to multiplex real-time reverse transcription polymerase chain reaction testing for typing of DENVs 1, 2, 3, and 4.

Results: Seroprevalence of dengue and CHIKV was reported to be 23.39% and 20.65%, respectively. Maximum number of cases were reported in the age group of 21–40 years. Common clinical presentations in dengue patients were fever, myalgia, headache, and nausea/vomiting whereas in CHIKV, the most common symptoms were fever, myalgia, and joint pains. On molecular surveillance, DENV-2 was detected in maximum cases (73.33%) followed by serotype 3 (11.66%).

Conclusion: Dengue and CHIKV infections continue to co-exist and there is substantial overlap in their clinical presentation. Simultaneous diagnosis of both viruses will help in evaluation, appropriate treatment, and prophylactic measures. Surveillance of DENVs needs to be closely monitored for the emergence of new serotypes.

Keywords: Dengue, Chikungunya, Coinfection, Dengue serotypes.

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INTRODUCTION

Vector-borne diseases account for over 17% of all infectious diseases causing more than 700,000 deaths annually and distribution of these diseases is determined by complex dynamic of environmental and social factors [1]. Dengue (dengue virus serotype [DENV]) and chikungunya (CHIKV) are rapidly spreading vector-borne diseases becoming a global public health issue due to their expanding geographical distribution. DenV belongs to the flaviviridae family while CHIKV belongs to family togaviridae; however, both viruses are made up of positive sense single-stranded RNA genome and share similar transmission vector, the Aedes mosquito species, and the epidemiology of both viruses is temporally and spatially related [2,3]. Although these viruses are geographically restricted, they are regularly carried from areas where they are endemic or epidemic to new areas where the population is immunologically naïve [4].

Dengue is the leading cause of death in the world and has been prevalent in nearly all the tropical and subtropical regions of the world. An estimated 390 million cases a year occur worldwide and infections are reported over 100 countries in the Asian-pacific region, America, Middle East, and Africa [5]. DENV has been categorized into four antigenically related serotypes DENV 1–4 and fifth serotype has also been reported in the past decade. Molecular and serosurveillance demonstrated the circulation of all DENV 1–4 serotypes from different geographical areas of India and can cause diseases ranging from subclinical infection to severe dengue such as dengue hemorrhagic fever and dengue shock syndrome [6,7]. Chinkungunya is emerging tropical infection that causes an acute illness with fever, rash, and severe arthralgia. Dengue and CHIKV case fatality rate is around 0.5–3.5% and 0.1%, respectively [3]. Since these viruses have common vector, similar clinical features, and geographic distribution, it is difficult to differential two infections. CHIKV fever is often misdiagnosed as dengue and true burden of CHIKV viral infection is missed in dengue-endemic areas [8]. Simultaneous infection with both viruses increases case fatality rate and complicates the diagnosis and further course of treatment. Many outbreaks of CHIKV have been reported from 2010 to 2022 in Asian countries with documented DENV cases which allow Aedes to become the carrier of both viruses and transmit the disease. There are isolated reports on DENV and CHIKV coinfection in India that has been reported in many states since 1964 [7]. The prevalence of coinfection documented in various studies varies from 0.9 to 19% [8]. Since there is no specific antiviral treatment for dengue and CHIKV, early and accurate diagnosis and distinction between dengue, CHIKV from other febrile illness could help identify patients, facilitate appropriate treatment, and calculate true burden of distribution and coinfection of these arboviruses. Continuous molecular surveillance of DENV serotypes is important to monitor circulating serotypes, and the mutations that can alter the diagnostics [6].

As there are multiple serotypes of dengue virus circulating concurrently with CHIKV virus, the present study was conducted to detect seroprevalence of dengue and CHIKV virus along with their coinfection and prevalent circulating DENVs.

METHODS

This cross-sectional study was carried out at the Department of Virology at Government Medical College and Hospital, Amritsar, from July 2023 to December 2024. The study included indoor and outdoor patients presented with acute febrile illness within 1–14 days, (≥38°C), falling in the probable case of dengue or CHIKV with or without warning signs as defined by the World Health Organization/Integrated Disease Surveillance Programme [7]. Patients presenting with upper respiratory tract symptoms were excluded from the study.

Approximately 5 mL of blood was collected in plain vacutainer from each patient with acute febrile illness under strict aseptic universal precautions and transported to laboratory at 4°C at the earliest. All the samples were properly labeled and particulars of the patient along with case history, demographic characteristics, comorbidities, clinical features, and evolution of symptoms were recorded on a designed pro forma and enclosed with samples. The samples were handled in biosafety level-2 facility. Blood was centrifuged and sera were separated for serological and molecular testing. Samples were stored at 4°C for maximum 72 h and at −70°C if needed to be stored for more than 72 h.

Serological testing

The serum samples were subjected to enzyme-linked immunosorbent assay (ELISA) for Dengue NS1 Ag (Qualisa Microwell Enzyme Immunoassay by Qualpro Diagnostics), Dengue MAC (dengue immunoglobulin M [IgM] ELISA kit, TRUSTwell by Athenese-Dx), and CHIKV MAC ELISA (Chik IgM ELISA Kit, TRUSTwell by Athenese-Dx). Test protocol was followed as per manufacturer's instructions, and optical density value was measured at 450 mm and the results were interpreted as described in the kit.

Molecular testing for dengue serotype detection

Further, samples which were positive for NS1 antigen were subjected to multiplex real-time reverse transcription polymerase chain reaction (RT-PCR) testing for typing of DENVs 1, 2, 3, and 4. Automated extraction of RNA was done on Kingfisher Flex (Thermoscientific) using MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit (Applied Biosystems). The extracted RNA was stored at −80°C until use. Serotyping of dengue virus was done by amplification assay using real-time PCR by TRUPCR DENV serotyping kit on BIO-RAD C1000 Thermal Cycler. The limit of detection of serotyping kit was 10^3 copies/mL. The thermocycling conditions were fed in the thermocycler and interpretation was done according to the kit insert. All reactions were run along with one positive and one negative control. Internal control was already integrated to check PCR inhibition. All clinical samples exhibited internal control reaction curves that cross the threshold line at or before 27 cycles, thus indicating that sample was of adequate quality. If positive control is amplified in all channels and negative control did not amplify in all channels, the assay was considered to be working. Different dengue serotypes in this assay were checked as they emit different fluorescence. The assay runs for 45 cycles, amplification beyond 40 cycles was not considered for interpretation, and the cutoff was 40 Ct.

Statistical analysis

All statistical calculations were done using the Statistical Package of the Social Sciences Version 21.

RESULTS

A total of 1702 samples from clinically suspected patients were received at the department of virology from July 1st to December 31st, 2023 to detect dengue and CHIKV viral infection. Out of total samples received for dengue (1073), 464 were subjected to dengue NS1 antigen, 609 to dengue IgM ELISA, and 581 were tested for CHIKV virus by performing ELISA. Overall seroprevalence of dengue was found to be 23.39% (64-NSI Ag positive and 187-IgM ELISA positive). CHIKV virus was detected in 120 cases with seroprevalence of 20.65%. Confection of dengue and CHIKV was also reported in 08 (1.9%) cases (Table 1).

The age-wise distribution showed maximum dengue-positive cases belonged to the age group of 21–40 years (43.82%). The age of the positive dengue cases ranged between 8 days and 85 years with the mean age and standard deviation of 37.90±55.49. Gender-wise distribution revealed more seropositivity of dengue among males (60.15%) as compared to females (39.86%). The range of age for males was 8 days–85 years with mean age and standard deviation of 38.08±17.55. The age range for females was 6 months–74 years with mean age and standard deviation of 37.70±17.65. A statistically significant difference was not found between the age of females and males (p=0.869).

Similarly, among CHIKV, maximum positive cases were also reported in age group of 21–40 (31.66%). The range of age of the positive CHIKV cases was between 3 years and 88 years, and the mean age and standard deviation of the positive cases were 41.28±18.64. The positivity of CHIKV was more among females (56.66%) as compared to males (43.33%). The ratio of males to females was 1.3:1 (Fig. 1). The age range for female cases was 3–80 years with mean and standard deviation of 39.56±17.21. The age range for male cases was 5–88 years with mean and standard deviation of 43.43±20.23. A statistically significant difference was not found between the age of females and males (p=0.300).

Fever and myalgia were present in all (100%) cases of dengue and CHIKV. Other common symptoms of dengue infection were chills and rigor (96.8%), headache (95.6%), nausea and vomiting (84.8%), and skin rashes (78.8%). Apart from fever and myalgia, CHIKV cases showed joint pains (91.0%), chills and rigor (90.0%), and headache (84.1%), as predominant clinical manifestations (Table 2).

The seasonal distribution of dengue and CHIKV cases is shown in Fig. 2. Maximum seropositives were detected in monsoon and post-monsoon

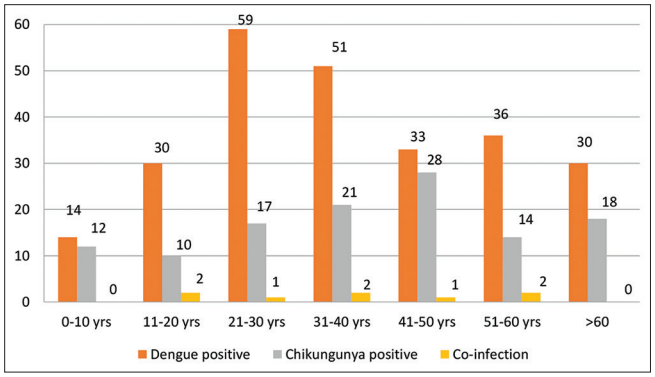

Fig. 1: Age-wise distribution of dengue and chikungunya cases (total dengue cases, n=251, total chikungunya cases, n=120, coinfection cases, n=8)

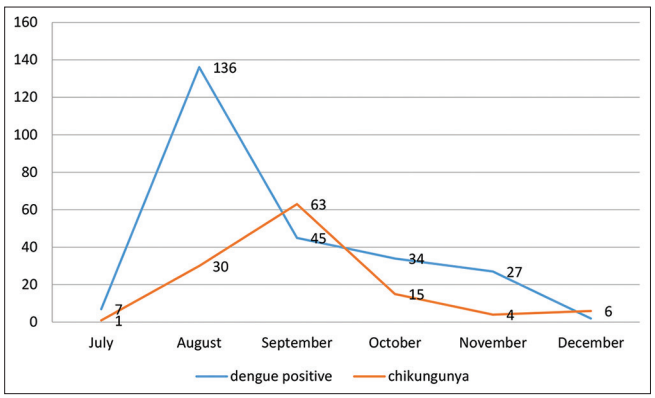

Fig. 2: Seasonal distribution of dengue and chikungunya cases (total dengue positive cases, n=251, total chikungunya positive cases, n=120)

Table 1: Seroprevalence of dengue and chikungunya infection

Samples tested	Dengue NS1 Ag ELISA (n)	Dengue IgM ELISA (n)	Chikungunya IgM ELISA (n)
Positive	64 (13.8)	187 (30.7)	120 (20.7)
Negative	400 (86.2)	422 (69.3)	461 (79.3)
Total	464	609	581
Odds ratio (CI)	0.36 (0.26–0.49)	2.76 (2.02–3.79)	1.70 (1.30–2.21)
p-value	<0.0001	<0.0001	0.0001

n=number of cases, *P*<0.05=significant, number of cases are mentioned in %frequency, CI=95% confidence interval. ELISA: Enzyme-linked immunosorbent assay, IgM: Immunoglobulin M

Table 2: Clinical profile of dengue and chikungunya cases

Clinical presentation	Dengue cases (n=251) (%)	Chikungunya cases (n=120) (%)	Co-infection (n=8) (%)	p-value
Fever	251 (100)	120 (100)	8 (100)	-
Chills/rigors	243 (96.8)	108 (90.0)	8 (100)	0.018
Headache	240 (95.6)	101 (84.1)	8 (100)	0.000
Myalgia	251 (100)	120 (100)	8 (100)	-
Joint pain	190 (75.6)	110 (91.0)	8 (100)	0.000
Nausea/Vomiting	213 (84.8)	41 (34.1)	7 (87.5)	0.000
Skin Rash	198 (78.8)	42 (35.0)	6 (75.0)	0.000
Thrombocytopenia	41 (16.3)	1 (0.8)	1 (25.0)	0.000
Bleeding diathesis	14 (5.6)	0	0	0.024
Retro-orbital pain	9 (3.5)	3 (2.5)	1 (25.0)	0.315
Ascites	6 (2.4)	0	0	0.904
Pleural effusion	4 (1.6)	0	0	0.772

n=number of cases, *P*<0.05=Significant, number of cases are mentioned in % frequency, *P* value calculated by one-way analysis of variance

season. More than 80% cases of dengue and 90% cases of CHIKV were reported from the months of August to October. Dengue cases showed peak in the month of August (54.4%), while CHIKV (52.5%) peaked in the month of September.

Out of 63 NSI Ag-positive samples, 60 were subjected to multiplex RT-PCR for typing of DENV. Our results demonstrated that DENV-2 serotype was predominant while the patients were also found to be infected with DENV-3 serotype. Monotypic DENV-2 was detected in 44 (73.33%) cases and DENV-3 in 7 (11.66%) cases. No cases of serotype 1 and 2 were detected in this region of study (Fig. 3).

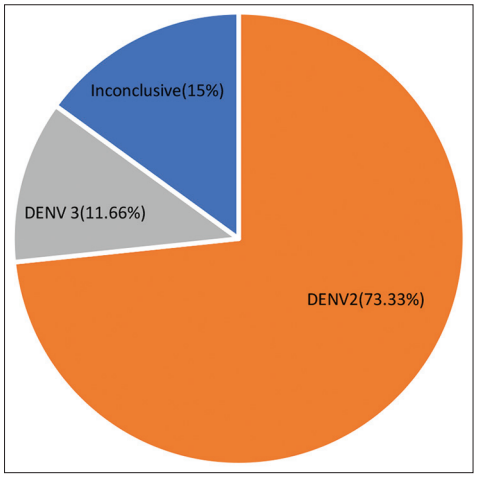

Fig. 3: Serotyping of dengue virus serotype isolates

DISCUSSION

Viruses are infectious agents that replicate only inside the body and the main ways of their transmission are through insects, by coughing and sneezing, by fecal-oral route, and by sexual contact [9]. Tropical arboviral infections are the most common cause of morbidity, represent a constant threat, have social and economic impacts on the population in endemic areas, and are further spreading beyond tropical areas [10]. Dengue and CHIKV virus infection is a disease transmitted through an arthropod vector known as an arbovirus. Two-fifths of the world's population is at risk from dengue sickness, according to the World Health Organization [11]. Every year, Indian states witness cyclical epidemics of dengue and CHIKV and that too with increasing numbers, showing that these viruses have adapted well to Indian climate and high population densities of vector, together with unplanned development, climate change have all contributed to the increase in vector-borne diseases.

In our study conducted over the period of 6 months, the seroprevalence of dengue and CHIKV was reported to be 23.39% and 20.65%, respectively (Table 1). Our findings are comparable to the prevalence rate of dengue (24.6%) documented by a similar study conducted earlier in Punjab in 2018 [12]. While studying the Nation wide data on epidemiology of dengue fever in India, Murhekar *et al*. also noted seropositivity rate of 28.4% [15]. A recent review on the epidemiology of CHIKV has analyzed that the prevalence of CHIKV in India ranges between 18 and 36% [16]. CHIKV virus has re-surged in India since 2010 after E1-A226V mutation together with adaptation to vector, *Aedes albopictus* which has led to the evolution of a new version of CHIKV virus [15]. Another interesting survey done by Palewar *et al*. over the period of 7 years reported alternate dominance in seropositivity of dengue and CHIKV over these study years. It has also been studied that there is drastic increase in seropositivity of CHIKV from 2016 (23.66%) onward as compared to 2014 (6.56%) and 2015 (7.29%) [16].

In our study, dengue and CHIKV positivity were predominantly seen in age range of 21–40 years (Fig. 1) 43.82% and 31.66%, respectively. Our findings correlate with the study of Neralwar et al. who reported 47.4% of dengue cases and Modi et al. reported 35.09% [15,16]. CHIKV cases from age group of 21–40 years. Study done by Pooja et al. in 2017–2018 also supported these findings [19]. Although dengue affects individuals of all ages and genders, more positive cases belong to young age group or working population in the productive phase of life who get exposed to mosquitoes more often as they go out for work. Adults in the age group of 21–40 are more prone to these arboviral infections as cited by many other authors [7,20,21]. Our findings are also consistent with the meta- analysis of published studies which reported the mean age of dengue and CHIKV-infected patients 27.79 years and 32.22 years, respectively [9].

Epidemiologically, working age group of 31–50 years was more infected with CHIKV, as seen by recent study from another author [22]. Males (60.15%) were predominately infected with dengue in our study, which correlates with the findings of Pooja et al. (55.6% males) and Neralwar et al. (63.19%) [15,17]. Male predominance has also been reported by many other studies from various parts of India [7,12] which could be due to greater exposure of males to outdoor activities, traveling, and different healthcare-seeking behavior of males and females. However, for CHIKV infection, females (56.66%) outnumbered males in our study. Similar gender data have been published by Balasubramaniam et al. and Chattopadhyay et al. who reported higher rates of CHIKV infection in females [23,24]. Pooja et al. have also seen gender shift in their study reported male predominance in 2017 and both sexes equally affected with CHIKV infection in 2018 [17]. Epidemiological parameters such as age and sex distribution of arboviral infections, similar to our figures, have also been reported from studies done from other states of India [25,26].

Dengue and CHIKV coinfection were also reported in 8 cases (1.9%). All cases with dual infection presented with fever, headache, and joint pains. Previous studies using serological methods had the prevalence of coinfection ranging from 0.9 to 19% [8]. Mamdi *et al*. recorded coinfection rate of 2.06% and all the patients in their study had fever, headache, joint pain, and thrombocytopenia [27]. In terms of clinical outcome, few studies have documented the severity of dengue and CHIKV coinfection; however, others indicated that neither symptoms nor clinical outcome was exacerbated by coinfection relative to monoinfection [3]. Palewar *et al*. also reported 5.79% cases infected with both dengue and CHIKV contributing to either coinfection or cross-reacting antibodies [16]. Both pathogens are transmitted by the same Aedes spp. mosquitoes and the epidemiology of both infections is temporally and spatially related [3]. Further studies and large sample size are required to understand the clinical profile of dual infections.

Fever and myalgia were seen in all dengue and CHIKV-positive cases (Table 2). Other than fever, the most common symptom in dengue cases was chills/rigors (96.8%), headache (95.6%), nausea/vomiting (84.8%), and skin rashes (78.8%). Thrombocytopenia and bleeding diathesis were seen in 16.33% and 5.6% cases, respectively. All (100%) CHIKV-positive cases had fever and myalgia; joint pain was also common clinical manifestation reported in 91.0% of patients. Skin rashes were reported in only 35% of patients and thrombocytopenia in 01 (0.1%). Singh *et al*. also reported fever (100%), headache (58.8%), nausea/vomiting (66.7%), and myalgia (48.1%) as common symptoms; joint pain (22.6%), skin rashes (19.6%), and bleeding diathesis (16.7%) were also reported. Fever, headache, and joint pain were predominant manifestations seen in all CHIKV patients studied [8]. Badoni *et al*. reported that the maximum cases of dengue and CHIKV-positive cases have symptoms of fever, chills, arthralgia, and headache [7]. Similar findings were reported in many studies across country done on the clinical profile of dengue and CHIKV [9,27].

The transmission of dengue increases in the monsoon and post-monsoon season as reported by various other authors [27]. We reported maximum cases of dengue (85.6%) and CHIKV (90%) from August to October (Fig. 2), the monsoon and post-monsoon period in northern states of India. Kausar *et al*. reported maximum number of cases (35.4%) in the month of September and October [28]. These months are more favorable for vector to survive and breed due to moisture, stagnation of rainwater, frequent water clogging, and agricultural activities [7]. Rising of cases during monsoon period had been reported in previous studies from India [8]. The number of cases increases because higher humidity lengthens the life span of mosquitoes and increased temperature shortens the extrinsic incubation period. Rainfall, humidity, and optimum temperature conditions are required for the breeding of mosquitoes and frequent water logging because of poor sewage system contributes to seasonal epidemics of dengue and CHIKV every year in Punjab. This seasonal pattern in our study is comparable to other Indian studies [16].

Four antigenically different dengue serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) are known to cause infection in human and all four DENV serotypes have been circulated in India [29]. In our study, 60 NS1 Ag-positive samples were subjected to multiplex real-time PCR to detect dengue serotype (Fig. 3). DENV-2 serotype was detected in maximum cases (73.33%) followed by DEN3 serotype (11.66%). No case of DEN V1 and DENV-2 was reported in our study. Similar data published from Northwestern India by Gupta *et al*. reported maximum cases infected with DEN V2 (34%) followed by DENV-3 (26%) [30]. The serotypes and genotypes of DENV were investigated by Alagarasu *et al*. and revealed that all the DENVs were circulating in India with differences in prevalence across the country [31]. Another study on serotype and genotype diversity on dengue viruses circulating in India reported circulation of multiple serotypes, and DEN V2 (44%) was most common followed by DENV-3 (3%), DENV-1 (16%), and DENV-4 (6%) [32]. Since DENV-2 serotype is the prevalent serotype reported from many parts of the country and this reiterates the requirement of dengue vaccine with high efficacy against DENV-2. Furthermore, data with respect to the hereditary arrangements of all the four serotypes, both during and between dengue pandemics, would be of significant incentive to figure epidemics [33] and can help estimate the clinical and laboratory trends of different serotypes. Molecular surveillance of DENV serotypes will also create database that will further help for the prediction of DENV outbreaks. Concurrent infection with more than one serotype is not seen in our study but simultaneous infection with two or more serotypes in a single person or concurrent infections ranges from 2.5 to 30% reaching 40–50% in hyperendemic areas [34].

CONCLUSION

Since the clinical presentation of DENV and CHIKV is similar, the CHIKV infections may go undiagnosed in dengue-endemic areas. Moreover, the two viruses may coexist in the same host and their clinical outcomes may be different. To understand the true extent of dengue and CHIKV infection, clinically suspected cases should be tested simultaneously for both viruses in endemic areas which would help in calculating the true burden of these arboviral infections. The surveillance of DENVs also needs to be closely monitored for circulating emergence of new serotypes. The genetic characterization of arboviruses, dual viral infections, and their correlation with clinical outcome will provide important information for further investigations, treatment, and management of patients. Molecular surveillance at different geographical locations will help to develop potential vaccine candidates.

CONFLICT OF INTEREST

None.

SOURCE OF SUPPORT

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