Bioresearch Communications
Volume 05, Issue 01, January 2019
Journal Homepage: www.bioresearchcommunications.com

Original Article

First dominant co-circulation and simultaneous co-infection of chikungunya and dengue viruses in Dhaka, Bangladesh during 2017

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ABSTRACT: Independent outbreaks of dengue virus (DENV) infection and sporadic cases of chikungunya virus (CHIKV) have been reported in Bangladesh on several occasions in the past. However, a massive febrile outbreak was observed in Dhaka city in summer 2017 and many cases turned negative for DENV. As there was high clinical suspicion of chikungunya, routine screening of serum from 1688 febrile patients were done for simultaneous detection and differentiation of chikungunya and dengue viruses by real time reverse transcriptase PCR. Interestingly, we found 627 (37.14%) cases of chikungunya and 277 (16.4%) cases of dengue and 11 (0.65%) cases of co-infection with both the viruses. This is the first report of dominant co-circulation of dengue and chikungunya and co-infections with dengue and chikungunya virus in Dhaka in a season and warrants widespread surveillance of both the viral infections in the country.

Keywords: Co-circulation, co-infection, chikungunya virus, dengue virus, RT-PCR, Bangladesh

Article History
Received: 30 August 2018
Accepted: 29 November 2018

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Citation : Mizanur Rahman , Rummana Rahim , Abu Hasan , Abu Sobhan Murad , Malay Biswas ; First dominant co-circulation Biores Comm . V5-(1) 649-654.

INTRODUCTION

Chikungunya virus (CHIKV) and dengue virus (DENV) are arboviruses currently circulating in Southeast Asia, Central and West Africa, the Pacific islands, and the Americas, and their simultaneous transmission can take place[1]. DENV and CHIKV co-infections have been reported from 13 of 98 countries/territories to which the viruses are endemic [2]. Although Bangladesh has had constant dengue transmission for >20 years, the first cases of CHIKV infection were reported in 2008 [3]. After few infrequent outbreaks between 2009 to 2015 a massive CHIKV outbreak was observed in Dhaka city in 2017 [4]. As the symptoms associated with the acute phase of dengue mono-infection are often indistinguishable from those presented by patients with chikungunya infection [5], confirmatory laboratory diagnosis is required for appropriate treatment recommendation. Again, most patients present during the acute, febrile-phase of the disease when antibody titers are typically below the level of detection limits of serological approaches, molecular methodologies to detect viral RNA are highly advantageous to detect and differentiate between co-circulating arboviruses and thus may facilitate rapid diagnosis and appropriate treatment. This prompted us to use one step real time reverse transcriptase PCR (RT-PCR) method to detect and discriminate CHIKV and DENV in blood during suspicious clinical symptoms. We have found 627 cases of CHIKV and 277 cases of DENV and 11 cases of co-infection with both CHIKV and DENV out of 1688 febrile patients visited in Apollo Hospitals Dhaka during 29 June 2017 to 31 March 2018.

MATERIALS AND METHODS

Patients and clinical specimens and ethical approval

Chikungunya dengue outbreak study was approved by the Research and Ethical Practices Committee of Apollo Hospitals Dhaka (approval number ERC 16/2017-2). 3 ml whole blood sample from adult and 0.5 ml to 1 ml from pediatric patients having clinical suspicion of either chikungunya or dengue were collected in plain vacutainer (red top) by phlebotomist of Apollo Hospitals Dhaka, Bangladesh during June 29, 2017 to March 31, 2018. Serum were separated and stocked at -80˚C until RNA was extracted.

RNA extraction

Viral RNA was extracted from 200 ul of serum following kit manufacturer's protocol (QIAamp MinElute Virus Spin Kit, Qiagen, Germany) and stored at -80˚C if not used immediately. For individual sample 25 µl protease was taken in 1.5 ml microcentrifuge tube and then 200 µl plasma, 200 µl lysis buffer containing carrier RNA (6.2 µl carrier RNA in 220 µl lysis buffer), 4 µl internal control were added sequentially in the tube. After brief vortexing the tube was incubated at 56°C for 15 minutes. Tube was then spinned down and 250 µl of 100% ethanol was added and vortexed and incubated at room temperature for additional 5 minutes. After spinning, the mixture of the tube was transferred to the QIAamp MinElute colum in a 2ml collection tube. Centrifugation was done at 8000 rpm for 1 minute & the flow through was discarded and column was transferred in a new collection tube. Two subsequent washing steps were performed by using wash buffer 1 & 2 (500 µl of each) & centrifugation was done at 8000 rpm for 1 minute & the flow through was discarded and column was transferred in a new collection tube. Then 500 µl of 100% ethanol was added again to the column and centrifuged at 8000 rpm for 1 minute & the flow through was discarded and column was transferred in a new collection tube. The blank centrifuge was done at 14000 rpm for 3 minutes. The column was transferred to 1.5 ml microcentrifuge tube & was allowed to incubate at 56°C for 3 minutes with open lid. Finally, 50 µl of elution buffer (Buffer AVE) was added to the column and incubated at room temperature for 1 minute. To obtain the final elution, centrifugation was done at 14000 rpm for 1 minute (Source: QIAamp MinElute Virus Spin Handbook- 04/2010, page: 17, 19-21, catalogue no. 57704).

Multiplex Real time reverse transcriptase PCR

Then one step reverse transcriptase real time PCR for the simultaneous detection of chikungunya and dengue virus was done by CE-IVD approved commercial kit from FTD (Fast Track Diagnostics, Luxembourg). 15ul PCR master mix containing 12.5 ul buffer, 1.5ul primer-probe mix and 1ul enzyme is prepared for each sample, positive control and negative control and then 10ul of the extracted RNA from samples, positive control and the extracted negative control is added, respectively. Then it is briefly mixed up and transferred the amount in 0.1ml PCR tube and placed the tube on thermocycler, Rotor Gene Q (Qiagen, Germany). According to kit manufacturer's instruction thermocycler is programmed which is briefly as hold at 50˚C for 15 minutes, again hold 1 min at 94˚C, then 40 cycles of 8 second at 94˚C and 1 minute at 60˚C. Each run was performed with a negative control (no template) and positive controls for CHIKV and DENV. Signal was acquired at 60◦C, and analysis was performed on the linear scale. Thresholds were set manually on each run. For both targets, any exponential curve crossing this threshold was considered positive. Fluorescence curve detected in Orange Channel are for amplification of Chikungunya virus and fluorescence curve detected in Green Channel are for amplification of Dengue virus and red channel for internal control.

RESULTS

Epidemiological findings

1688 febrile patients between June 29, 2017 and March 31, 2018 were screened for CHIKV and DENV infections, of whom 627 (37.14%) were CHIKV-positive, 277 (16.4%) DENV positive, and 11 (0.65%) both CHIKV positive and DENV positive. There were 944 men and 743 women (sex ratio, 1.27), with age range, 1 day – 96 years. Gender distribution and age group data analysis were done further and shown in figure 1 and table 1, respectively.

Table 1. Spatial (area) distribution of RT-PCR confirmed cases of chikungunya and dengue in Dhaka

Area	No. of CHIKV+ cases	No. of DENV+ cases
Bashundhara	152	76
Baridhara	30	17
Gulshan	55	26
Banani	27	7
Uttara	146	48
Rampura	22	21
Badda	28	5
Dhanmondi	10	2
Malibagh	15	3
Mirpur	39	23
Mohakhali	19	0
Mohammadpur	4	4
Khilkhet	8	10
Khilgaon	8	1
Shahbag	3	1
Old Dhaka	26	4
Turag	2	2
Tejgaon	3	3
Gazipur	6	6
Savar	4	1
Narayangonj	3	2
Norsingdi	2	1
Munsigonj	1	0

CHIKV+, chikungunya virus positive; DENV+, dengue virus positive; RT-PCR, reverse transcriptase polymerase chain reaction.

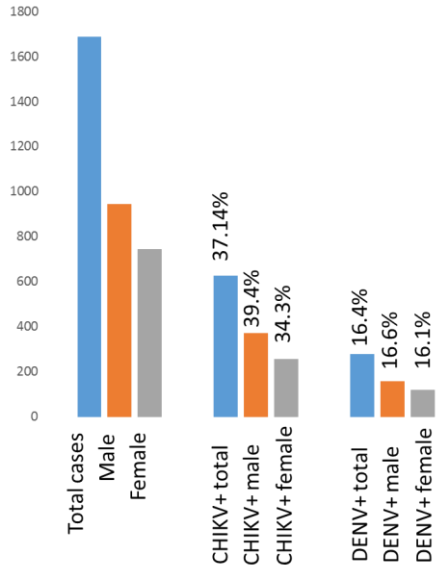

Figure 1. Gender distribution of total febrile cases, chikungunya and dengue cases with frequency in 2017

Out of 627 CHIKV-positive patients 372 (39.4% frequency) were men and 255 (34.3% frequency) were women (sex ratio, 1.45); out of 277 DENV–positive patients 157 (16.6% frequency) were men and 120 (16.1% frequency) were women (sex ratio, 1.3); and the out of 11 co-infected patients 6 were men and 5 were women (sex ratio, 1.2), with age range, 7 years–65 years. Most of the patients were from Dhaka (table 1) with the dominance from Bashundhara, Uttara, Gulshan, Baridhara, Rampura, Banani, Mirpur, Mohammadpur and old Dhaka and few (28) patients were from out of Dhaka Division (not shown in table 1). Both chikungunya and dengue is found in patients visited from almost all area of Dhaka City.

Co-circulation of chikungunya and dengue

At the beginning of summer 2017 the number of febrile patients visited Apollo Hospitals Dhaka, a tertiary care hospital in the country started to increase and the number of patients increased very significantly at May. Serum from these patients were mostly dengue NS1 negative and there was high clinical suspicion of chikungunya. However, laboratory diagnosis of chikungunya was not possible till end of June due to unavailability of reagents. From beginning of July we started to detect and differentiate CHIKV and DENV by commercial single step multiplex reverse transcriptase PCR. Among the 1,688 acute phase serum samples from 29 June 2017 to 31 March 2018, 627 (37.14%) were confirmed by qPCR as CHIKV and 277 (16.4%) as DENV positive. Highest number (285) of CHIKV positive cases were found in July and then gradually decreased (figure 2).

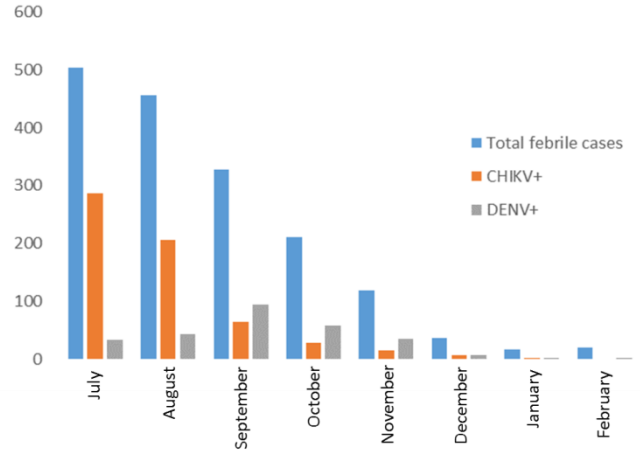

Figure 2. Temporal distribution of chikungunya and dengue in Dhaka

CHIKV-positive cases were 10 times more numerous than DENV positive cases during the month of July, 4.7 times more in August, and then DENV positive case number increased than CHIKV positive cases. Highest number of DENV positive cases were found in September and it started to decrease from October but still more than two fold than the CHIKV positive cases found in this month. The year ended with equal number (six) of both the cases of CHIKV and DENV and the season ended at March when no case of CHIKV and DENV was found. Throughout the season adult males were more affected than females and people of all age groups were affected (table 2).

Table 2. Distribution of RT-PCR confirmed cases of chikungunya and dengue

Age (year)	Suspected case no.	CHIKV+, No. (%)	DENV+, No. (%)	Co-infection No.
<1	33	6	5	0
1-10	447	105	63	2
11-20	173	47	39	0
21-30	183	72	42	2
31-40	233	108	48	2
41-50	208	77	46	2
51-60	120	58	17	2
61-70	152	86	11	1
>70	139	65	9	0
Total	1688	627(37.14%)	277(16.4%)	11(0.65%)

CHIKV+, chikungunya virus positive; DENV+, dengue virus positive; RT-PCR, reverse transcriptase polymerase chain reaction

Co-infection of 11 cases with chikungunya and dengue viruses

Both CHIKV and DENV positive 11 cases were detected by RT-PCR (table 1). Clinical data of these 11 co-infection cases were shown in table 3.

Table 3. Clinical symptoms of chikungunya and dengue co-infection cases

Patient (years)	Age	Sex (Days)	Fever (other than fever)	Symptoms
P1	65	F	2	Bodyache, Vomiting
P2	28	F	7	Weakness, Loss of appetite
P3	44	M	n/a*	Bodyache, Joint pain, Rash
P4	25	M	n/a*	Joint pain
P5	7	F	3	Itching, Loss of appetite
P6	51	F	1	Bodyache, Vomiting,
P7	36	M	4	Bodyache, Vomiting, Loose motion
P8	9	F	4	Vomiting, Vertigo, Loss of appetite
P9	54	M	3	Weakness, Loose motion
P10	43	M	2	Shortness of breathing
P11	36	M	4	Bodyache

* record not available

Six patients admitted in hospital and none had hemorrhagic or neurological complications. All had fever and duration of fever varies at time of visiting clinic. Five patients (P1, P6, P7, P8, P9) had gastrointestinal symptoms like vomiting, loose motion and loss of appetite. Except P1 DENV load was higher in these patients and there was relative lymphocytosis except P6 (table 4).

Table 4. Laboratory data analysis of chikungunya and dengue co-infection cases

Patient No.	TLC Value	Diff. Count (relative)	PLT count	CHIKV PCR Ct	DENV PCR Ct
P1	Decreased	Lymphocytosis	Normal	16.75	30.09
P2	Decreased	Lymphocytosis	Normal	19.75	20.33
P3	Normal	Neutrophilia	Normal	25.6	35.7
P4	Normal	Neutrophilia	Normal	28.96	35.95
P5	Normal	Lymphocytosis	Normal	13.76	35.36
P6	Normal	Neutrophilia	Normal	32.71	19.1
P7	Decreased	Normal	Normal	26.74	21.95
P8	Decreased	Lymphocytosis	Normal	30.89	19.7
P9	Decreased	Lymphocytosis	Reduced	30.82	25.65
P10	Normal	Neutrophilia	Normal	12.77	28.75
P11	Decreased	Normal	Normal	26.08	22.5

TLC-total leukocyte count, Diff.- differential, PLT-platelet, Ct- cycle threshold

Relative lymphocytosis is a common feature in dengue patients 6. Relative lymphocytosis was also seen in P2 and P9 and DENV load was seen higher in P9 but not in P2. Further, we have noticed that in majority of these cases relative lymphocytosis developed few days later than initial cell count at time of visit. Relative lymphocytosis also corresponded with decreased total leukocyte count except P5. Two (P3, P4) patients had joint pain (arthralgia) and relative neutrophilia and higher chikungunya viral load was seen (CHIKV PCR cycle threshold (ct) value appeared earlier than DENV PCR Ct value). No specific location of arthralgia was noted, and no relation was found between co-infection and sex (sex ratio, 1.1) or age (range, 7–65 years). Relative neutrophilia (P3, P4, P6, P10) was associated with normal total leukocyte count. Platelet count was normal in all co-infection cases except P9 whose platelet count decreased and DENV load was higher than CHIKV load. Though there is no clear cut association between symptoms and viral loads there are few observations, (a) patients with gastrointestinal symptoms had higher DENV load than CHIKV load (b) patient with joint symptom had higher CHIKV load than DENV load (c) relative lymphocytosis is more seen in cases where DENV load is higher than CHIKV load (d) relative neutrophilia is more seen in cases where CHIKV is higher than DENV load.

DISCUSSION

Bangladesh is within the 68 countries/territories reported the presence of vector species Ae. Aegypti and Ae. Albopictus for transmission of both CHIKV and DENV 2. Dengue is endemic in Bangladesh since 2000 7 and chikungunya is sporadically reported from 2008 8. There was a massive outbreak of chikungunya in Dhaka city, Bangladesh in 2017 9. However, there is no report of co-circulation and co-infection of CHIKV and DENV in the country and global maps generated from the compiled lists of the geographic distribution of both CHIKV and DENV and vectors showed lack of co-infection in Bangladesh though it is already showed in the surrounding countries 2.

The first cases of dengue-chikungunya co-infection were reported in Asia in Thailand by Nimmannitya et al. who detected four co-infected cases among 150

patients diagnosed with either dengue or chikungunya (2.6 %) in 1962; three co-infected cases out of 144 infected patients (2.1 %) in 1963; and 12 co-infected cases out of 334 infected patients (3.6 %) in 1964 (10). In 1964, co-infection cases were also reported in south India (11-12) during a spate of chikungunya epidemics spanning 1963–1973 13. It is also reported in Myanmar during 1970 – 1972. After that no reports were found of dengue-chikungunya co-infections for more than 30 years despite sustained CHIKV and DENV endemicity in Asia and Africa. Co-infection of CHIKV and DENV were again found in India, Srilanka, Madagascar, Malaysia in 2006 and it is continued and spread to more countries in South East Asia and Western Pacific region till today.

In terms of clinical outcome, only four studies have described the severity of dengue-chikungunya co-infection 14-17. Three studies indicated that neither symptoms nor clinical outcome were exacerbated by co-infection (relative to monotypic infection). Only Chahar et al. described a high rate of severe symptoms and poor clinical outcomes among co-infected patients 14. Here, we also did not find disease severity or any particular clinical manifestation associated with co-infection. We only notice that dengue-chikungunya co-infection rate is low as compared to the other surrounding countries.

Misdiagnosis of chikungunya as dengue (and vice versa) is reported previously 14. Misdiagnosis not only hampers epidemiological understanding of both the diseases but can profoundly affect the clinical outcome, specially, if dengue is misdiagnosed as chikungunya. Because, in such a case it risks inappropriate prescription of arthralgia-alleviating nonsteroidal anti-inflammatory drugs (often employed in treating chikungunya patients) which could lead to severe bleeding in patients with thrombocytopenia or dengue hemorrhagic fever 18.

In this report we provide first evidence of prevailing co-circulation of dengue and chikungunya and simultaneous co-infection with dengue and chikungunya virus in Dhaka and warrants widespread surveillance of both the viral infections in the country. Further, our report shows that molecular detection at time of clinical presentation will avoid misdiagnosis and may unveil true geographical extent of CHIKV and DENV and population at risk of infection.

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