A study presenting the analysis of co-infection of chikungunya and dengue virus in a microbiology laboratory at tertiary care hospital in western India

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Abstract
Coinfection means simultaneous infection by two or more virus. Dengue and Chikungunya are viral infections caused by RNA virus (arbovirus). The transmitting vector for both viruses is Aediesaegypti. Human co-infection with DENV and CHIKV have been reported in India since 1967. DENV and CHIKV share a number of similarities in clinical presentation which includes seasonal transmission cycle. This study was carried out in Virology Department, PDU Medical College, Rajkot. A total of 3810, 2122 and 1605 hospitalized patients were serologically screened for DENV, CHIKV and both, respectively between July and October 2017 by IgM Capture ELISA. Co-infection was defined as a positive IgM assay for both infections.60 (3.73%) were IgM positive for both DENV and CHIKV. 474 (12.44%) were mono-infected with DENV and 721 (33.97%) were mono-infected with CHIKV. Simultaneous infection with Dengue and Chikungunya viruses is having more severity than single infection. Early detection and time bound Notification of the positive cases help to reduce the morbidity and mortality rate and help the health authorities to execute effective measures for prevention and control of diseases. This will also help in increasing vigilance before any epidemic.

Keywords: Co infection, Dengue, Chikungunya, ELISA.

Introduction
In Microbiology, Coinfection is the dual infection in which there is simultaneous infection by multiple pathogens in a single host. Dengue, Chikungunya, Malaria, Filariasis are classic examples of infection transmitted by mosquito. They are major trouble on the maintaining health-care system. First outbreak of the Chikungunya virus occurred in 1963 in Kolkata,[1] after this several epidemics in different states of India.[2,3] Chikungunya appear again in many states of India in 2005 and by 2010, virus has been spread to more than 18 states within the India.[4] Currently Chikungunya has being reported as a coinfection with dengue in many parts of India. There are many studies in India showing the acute presentation of the chikungunya virus infection and long-term effects it. Still there are very limited studies representing the analysis of Chikungunya-Dengue co-infection.[5,6]

Dengue is an arboviral infection having endemic nature. Dengue is seenmainly in urban and the semi urban areas in various tropical regions and also the subtropical regions in the world. Severe dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) is the main complications of Dengue virus (DENV) infection. This is also responsible for many cases of acute febrile illnesses. Dengue virus infection is prevalent in our country, since 1996.

Dengue and Chikungunya are microbial infections caused by RNA virus (arbovirus). Transmitting vector for these viruses is Aedies aegypti. Dengue virus belongs to the group of arboviruses. Viruses in this group are being transmitted by arthropod vector which includes mosquito, ticks and flies. As the Dengue and Chikungunya viruses are transmitted by same vector, Epidemics with these viruses affect the same localities in many parts of South Asia.[7] co-infection with dengue virus (DENV) and chikungunya virus (CHIKV) are being reported in India Since 1967.[8]

Dengue Virus

Family of Dengue virus is Flaviviridae. DEN1 to DEN4 are four different types of dengue viruses. In recent years, 5th serotype DEN-5 has been discovered. Aedes aegypti followed by Aedes albopictus is the vectors which can spread the dengue infection. Aedes aegypti mosquito is nervous feeder which means it can bite more than 1 humans in a single feeding; Aedes albopictus is aggressive and concordant feeder which can finish its blood meal in single time. Principal reservoirs for this virus are Man and Aedes mosquitoes. Animals are not having any role in the transmission of the viruses.

Chikungunya Virus

Family of Chikungunya virus is Togaviridae. This virus is being transmitted by vector Mosquito which is Aedes aegypti. There is mutation in virus genome which is responsible for re emergence of the Chikungunya disease in few recent years. *New mutational Virus contains replacement of valine by Alanine in the 226 position of E1 glycoprotein gene.* New Vector – Aedes albopictus which is more infective in spreading the chikungunya disease.[9] DENV and CHIKV share a number of similarities in clinical presentation which includes seasonal transmission cycle.

Table 1: Assessment of clinical parameters of chikungunya and dengue diseases

Clinical parameters	Chikungunya	Dengue
Fever	Common	Common
Minor Bleeding	Common	Common
Tenosynovitis	Common	None
Rashes appear on	Day 1-4	Day 3-7
Myalgia	Possible	Common
Retro orbital Pain	Rare	Common
Platelets count	Normal	Decreased
Leukocyte count	Normal/Increased	Decreased
Hypotension	Possible	Common
Polyarthritis	Common	None
Reservoir	Human & Animal	Human

Objectives

To Study the effects of Co infection with Dengue virus and Chikungunya virus and to know the prevalence of co infection with the same viruses.

Materials and Methods

The study was carried out in Virology Department, PDU Medical College, Rajkot. A total of 3810, 2122 and 1605 hospitalized patients were serologically tested for dengue virus (DENV), chikungunya virus (CHIKV) and both dengue chikungunya virus for co infection, respectively. This study was carried out between July and October 2017. All tests were done by IgM Capture ELISA by using a commercial kit ie NIV IgM Capture ELISA Kit for DENV & CHIKV, Manufacturer: National Institute of Virology, Pune. Sensitivity and specificity of the kit is 97.94% and 96.98%, respectively has been reported[10]. On molecular basis we can confirm all positive cases by doing RT-PCR. Mono-infection is defined as positive IgM Capture ELISA for only one of these viral infections either with dengue virus or with chikungunya virus. Co-infection was defined as positive IgM Capture ELISA for both infection, dengue virus and chikungunya virus as well. Online reports of the results were sent to the respective District & State health authorities daily, weekly and monthly in Integrated Disease Surveillance Project (IDSP) format for further treatment and taking preventive measures.

Results

A total of 3810, 2122 and 1605 hospitalized patients were serologically tested for dengue virus (DENV), chikungunya virus (CHIKV) and both dengue chikungunya virus for co infection, respectively. This study was carried out between July and October 2017. All tests were done by IgM Capture ELISA.60 (3.73%) cases were IgM positive for both dengue virus (DENV) and chikungunya virus (CHIKV). 474 (12.44%) were mono-infected with dengue virus (DENV), means positive for dengue virus.721 (33.97%) were mono-infected with chikungunya virus (CHIKV), means positive for chikungunya virus. Dengue IgM, Chikungunya IgM and Co infection results are shown in Fig. 1 and 2. The salient clinical features of acute dengue infection and dengue with chikungunya co-infection and acute chikungunya infection are compared in Table 1. Leucocyte count was decreased, more commonly in patients with dengue infection as compared with chikungunya in which there was normal leucocyte count or an elevated leucocyte count. Platelet count was decreased (<150,000/µL) in 11 patients of chikungunya, 107 of dengue positive patients and 7 of was having co-infected patients. Analyses of the tested patients presented that majority of the infected patients were in the age group of 19-30 years followed by the 31-45 years of age group (Fig. 3) and Male are highly infected than Female (Fig. 4) as they are more active doing outside work so they get infected easily.

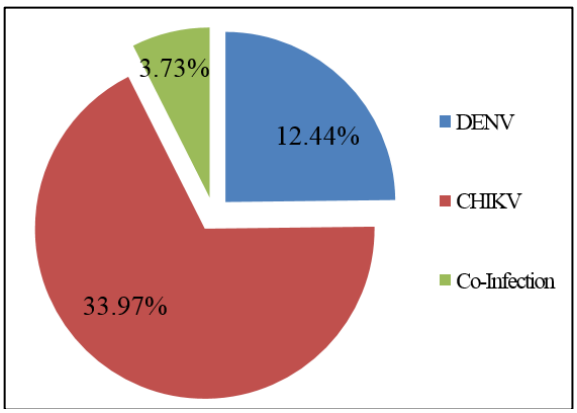

Fig. 1: Data showing positive results for DENV and CHIKV from clinical samples

Discussion

Mosquitoes like Aedes aegypti and Aedes albopictus are principal vectors for transmission of dengue virus (DENV) and chikungunya virus (CHIKV). Both the viruses are often present simultaneously in the mosquito and both can transmit infection to susceptible individual as co-infections after mosquito bite. Symptoms of both the diseases often overlap to each other as they are difficult to differentiate. Most common Clinical features during an acute phase are, fever, joint pain, bone pain, nausea, vomiting, headache, body ache and fatigue. In this study, all suspected patients either from OPD or IPD, both were tested for Dengue or Chikungunya infection in virology laboratory. Prognosis is different in both the cases in spite of having many common clinical parameters. Dengue can lead to major complications like hemorrhagic fever, shock and also including death while Chikungunya is mainly nonfatal disease limited to joint pain and fever only. As there are no any anti viral drugs are available, treatment is mainly based on supportive and nutritional care. Peak season for epidemics of these

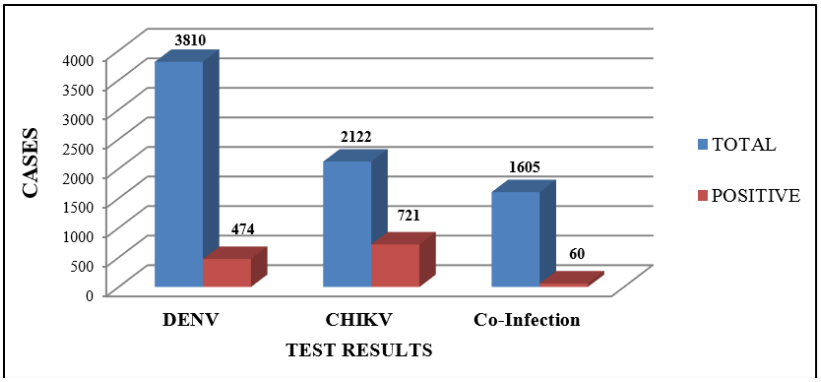

Fig. 2: Data showing positivity ratios results of clinical samples

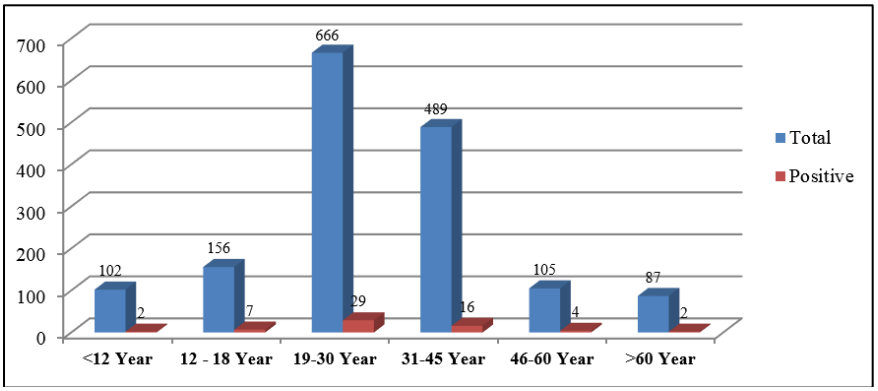

Fig. 3: Age wise distribution of study participants

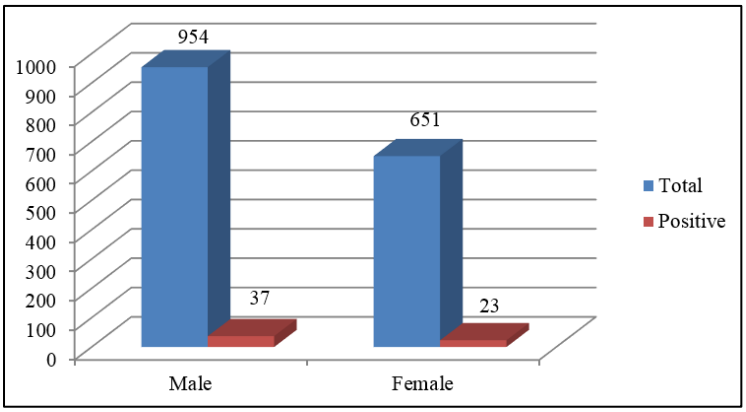

Fig. 4: Gender wise distribution info study participants

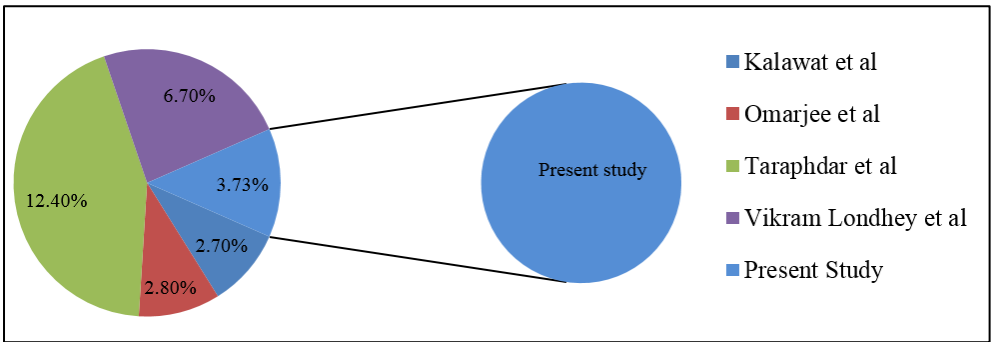

Fig. 5: Comparisons of results of various studies with same objectives

disease are mainly post monsoon period which is between first week of September and mid-October. During this time there are ideal environmental conditions which favour breeding places for vectors and thus increasing number of mosquitoes and clinical cases. There has been sameresultin the previous studies reported in India.[11] Fig. 5 represents the prevalence of co-infection by doing serological methods has been reported in the previous studies by Kalawat et al as 2.7%,[11] Omarjee et al[12] as 2.8%, Taraphdar et al[13] as 12.4%, Vikram Londhey et al[14] as 6.7%. In present study 3.73% cases are positive for both viruses, dengue virus and chikungunya virus, which is nearby similar with Kalawat et al and Omarjee et al. This study mainly aims the comparison of serological and clinical profile of dengue and chikungunya cases with other reference studies done so far.

Conclusion

This study showed the wide spread of co-infection with dengue and chikungunya virus which present in local areas of Rajkot and surrounding area in western India. Co infection with these both the Dengue and Chikungunya viruses are more severe than single infection clinically. More detailed studies are require for analysing the pathogenesis and complications of co-infection to detect severity and clinical outcome of co infection. Simultaneously safety measures are required to taken up by local authorities to reduce the chances of transmission of infection. Rapid detection of the positive case and immediate Notification of these cases to health authorities can help to decrease the morbidity and mortality rate for this kind of infections. Detailed studies on the same topics in future will also help us in taking precautions and preventive measures before outbreak.

Conflict of Interest: None.

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