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Co-infection with Dengue and Chikungunya Viruses

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Abstract

Dengue and Chikungunya fever are the arboviral infections that are endemic in tropical and subtropical regions. These two viral infections share common clinical symptoms. These infections are transmitted by a common mosquito vector so these viruses co-circulate in many geographical regions. Various clinical investigations, particularly from India and African countries have documented the dual infection with these viruses. However, the true disease burden of Dengue and Chikungunya dual viral infections is still not known because most of these studies involved a smaller patient group. Therefore, in depth investigations involving larger patient groups are needed to examine the complete pathogenicity and severity of the dual viral infections. The timely diagnosis of the pathogens and correlation of disease severity with mono or dual infections is essential for effective patient management. In addition, the detailed molecular and cellular mechanism of co-infection should be investigated to describe a complete picture of the interaction of two viral pathogens in the host cell. Further comprehensive studies of dual infections from the endemic regions will determine the epidemiological and evolutionary pattern of these emerging viruses. This data will also assist in designing and implementation of effective control measures.

Keywords: Chikungunya virus, Dengue virus, co-infections, *Aedes mosquito*

1. Introduction

Both Dengue and Chikungunya fever are arboviral infections of global importance. The two diseases share a common mode of transmission, i.e. through different species of mosquitoes. Therefore, these infections are normally present in the same geographical locations. Dengue virus belongs to family Flaviviridae and genus Flavivirus. It is a small enveloped RNA virus carrying a single stranded, positive-sense RNA genome of 10.6 kb in length. Dengue virus exists as four serotypes (DENV 1-4) which offer only transient cross protection to each other

[1]. Four to five genotypes are distinguished within each serotype of the dengue virus. The Chikungunya virus (CHIKV) belongs to the *Togaviridae* family and genus *Alphavirus*. It has a linear positive sense RNA genome of 1.8 Kb in length. Three genotypes (Eastern, Central South African [ECSA], West African and Asian) have been described for the Chikungunya virus [2,3]. Laboratory diagnosis of the two viral infections is done by virus isolation, genome detection (RT-PCR) and antibody detection (IgM or IgG ELISA). In addition, the antigen detection (NS1 ELISA) is also being used for diagnosis of Dengue viral infection. No licensed vaccine is available against these arboviral diseases. In addition, no antiviral drug has been developed against these viruses.

Due to many common clinical presentations, Chikungunya fever is often misdiagnosed with Dengue viral infection. As Dengue fever has a high incidence rate, the symptomatic patients are tested for Dengue virus only and rarely for Chikungunya viral infection. Thus the patients suspected with Dengue and/or Chikungunya virus infection should be tested for both the viruses especially in the endemic areas. This is essential for accurate and timely diagnosis of the viral infection that will assist in appropriate patient management. Therefore, regular surveillance for both the viruses should be done in the endemic areas. This will assist in the prediction and control of the outbreaks. The molecular characterization of the circulating strains of the Dengue and Chikungunya viruses is done by DNA sequencing followed by phylogenetic analysis. Description of the circulating strains is essential to study the epidemiology of these rapidly evolving viruses [4,5,6]. In addition, this information is essential for designing the strategies to control the epidemics.

2. Clinical manifestations

Both Dengue and Chikungunya viral infections have many common clinical presentations like high grade fever, headache, nausea, rashes and body pain. In case of a mild infection, the viral titre decreases in around 10 days and the symptoms subside because these are the self limiting infections. But when there is a severe dengue infection, it causes bleeding in DHF (dengue hemorrhagic fever) and/or shock caused by plasma leakage in DSS (dengue shock syndrome). The most prominent feature of Chikungunya infection is the severe joint pain which sometimes can persist for a few months to a year. A severe Chikungunya viral infection can cause neurological and optical manifestations. Thus, Chikungunya viral infection is usually non-fatal while Dengue fever may result in severe complications including death. Therefore, co-infection with the two viruses may result in disease with overlapping symptoms. Hence, the diagnosis and treatment of such patients become difficult. Therefore, this issue of clinical manifestations in case of dual infections with the two viruses should be addressed adequately. Hence, the timely diagnosis of the dual infections is essential for better patient management.

Limited investigations have studied the role of dual viral infections in clinical presentation of the disease. According to a recent investigation, patients having co-infection with the Dengue and Chikungunya viruses present a clinically severe disease with a high mortality rate when compared to mono-infection with these viruses [7]. The requirement of mechanical ventilation and blood transfusion was found to be higher in the co-infected patients. Chahar and colleagues also reported the involvement of central nervous system and hemorrhagic manifestations

in two out of six co-infected patients and one death [8]. In contrast, another study from Africa compared the clinical symptoms of 19 Chikungunya and Dengue virus co-infected patients and it was concluded that the co-infection was also not associated with any particular clinical manifestations [9]. Further elaborate investigations involving larger patient group are needed to understand the complete pathogenesis and severity of the dual viral infections.

3. The vector

The main vector for the transmission of Dengue and Chikungunya viral infection is *Aedes aegypti*. Another species of mosquito, the *Aedes albopictus* act as the secondary vector for the transmission of both the viral infections. The *Aedes albopictus* emerged as highly competent vector for the transmission of Chikungunya viral infection during the massive outbreak in 2004 in the Indian Ocean [10]. The *Aedes albopictus* appeared to be an efficient vector for replication of a mutated strain of Chikungunya virus with a change in Alanine to Valine at 226 position of the E1 glycoprotein (E1-A226V) during this epidemic [11]. The *Aedes albopictus* commonly known as the Asian tiger mosquito is a tropical and subtropical vector. The overall geographical distribution of *Aedes* mosquitoes has changed since this outbreak. The *Aedes albopictus* is now spreading to regions that were earlier inhabited by the *Aedes aegypti*. Consequently, the circulation patterns of the Dengue and Chikungunya viruses as well as the dynamics of the epidemics caused by these viruses are expected to be modified in future [12].

4. Dengue and Chikungunya virus co-infection in the vector

It has been postulated that two different viruses cause dual infection of a mosquito vector by consecutive bites of two different infected human patients or by a single bite of a co-infected patient. Furthermore, a concurrent viral infection in humans occur due to bite of a mosquito that is infected with both the viruses or bites with two different mosquitoes each infected with a separate virus. Limited investigations are available on the role of vectors in disease transmission because of lack of technical expertise and limited resources of vector surveillance in the endemic areas. *Aedes albopictus* has been shown to have the ability of getting orally co-infected with Dengue and Chikungunya virus [13]. The study reported that both Dengue and Chikungunya viruses were able to replicate simultaneously in the mosquito and have the ability to deliver concomitantly infectious particles of Dengue and Chikungunya virus in a single bite via saliva. In addition, a secondary infection with the Chikungunya virus could be introduced in mosquitoes that had a primary infection with Dengue virus. Another recent study showed simultaneous infection, dissemination and transmission of Dengue and Chikungunya virus in the two mosquito species, *Aedes aegypti* and *Aedes albopictus* [14]. In this study, groups of mosquitoes were orally infected with Dengue and Chikungunya virus simultaneously or sequentially. Mosquitoes were then tested for their potential to disseminate and transmit both the viruses simultaneously by quantitative RT-PCR. Simultaneous dissemination of Dengue and Chikungunya virus was detected in both the species of the mosquitoes. The authors observed a lower rate of dissemination of both the viruses when administered

simultaneously as compared to the sequential infection in which a significantly higher rate of dissemination of both the viruses was found.

Earlier studies have suggested that vector competence of the mosquito may depend on the type and the genetic makeup of infecting viral strain. An investigation from France stated that *Aedes albopictus* was more competent for Chikungunya virus as compared to the Dengue-2 virus. The virus replicated to high levels in the mosquito species and could be transmitted from mosquito as early as 2 days after ingestion of infected blood by the mosquito with 1000 viral RNA molecules in the salivary glands [15]. Similarly, another study from Thailand also concluded that the rate of multiplication and oral receptivity of the Chikungunya virus was faster in laboratory-bred *Aedes aegypti* as compared to the Dengue virus [16]. *Aedes albopictus* was proved to be an efficient vector for dissemination and transmission of the Chikungunya virus as compared to *Aedes aegypti* [10]. A single amino acid mutation in the Chikungunya virus genome (E1-A226V) resulted in increased fitness of the virus in *Aedes albopictus*. This probably caused enhanced transmission of the Chikungunya virus by *Aedes albopictus* which further resulted in the massive outbreak in the Indian Ocean. The complete mechanism of co-infection of mosquitoes with two different arboviruses should be investigated further using different viral strains. The elucidation of detailed molecular and cellular events involved in such co-infections will have an impact on prediction and control of the viral outbreaks.

5. Dengue and Chikungunya virus co-infection: *in vitro* studies

Co-infection of the two viruses has been studied *in vitro* also using *Aedes albopictus* C6/36 cell line [17]. In this study the DENV-3 and Chikungunya virus (ECSA genotype) isolated from the infected mosquitoes was used to characterize their co-infection. The duplex -RT-PCR (D-RT-PCR) technique was used to determine virus production. The D-RT-PCR was positive for both the viruses when an equal multiplicity of infection (MOI) or a higher MOI for Chikungunya virus was used. But when a higher titer of DENV-3 was used, the D-RT-PCR was positive only for DENV-3. Thus, the authors concluded that higher titer of Dengue-3 virus resulted in competitive suppression of the replication of Chikungunya virus. They also reported that both viruses' replications depend on virus titer and not on serial infection. Further elaborate investigations are needed to describe a complete picture of the interaction of the two viral pathogens in the host cell.

6. Dengue and Chikungunya virus co-infection in humans

Due to common vectors, Dengue and Chikungunya viruses can co-infect a human host. This has been documented in several studies from India and Africa (Table 1) (Figure 1 and 2). The first documented case of Dengue and Chikungunya virus co-infection in humans was reported as early as 1967. The two viruses were isolated from a patient seen in Vellore, South India [18]. The patient also showed an increase in antibodies against Dengue and Chikungunya virus in serial blood samples. Subsequently co-infection of Dengue and Chikungunya has been

reported in many studies from India, but the proportion of co-infected cases vary in different studies ranging from 0.1 to 23% (Figure 2).

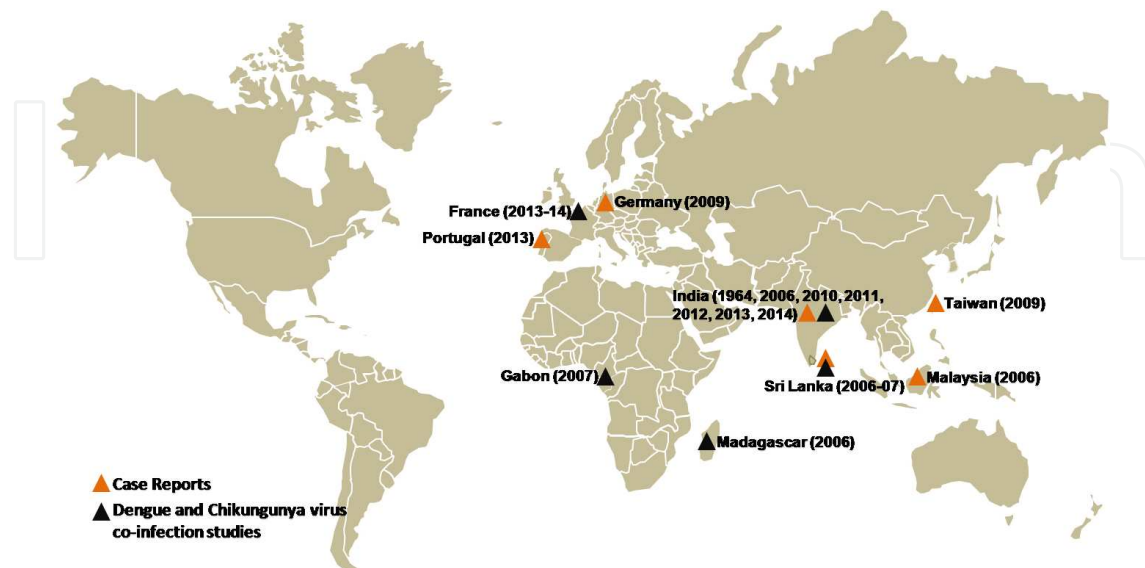


Figure 1. The world map showing co-infection with Dengue and Chikungunya viruses in different geographical regions. The freely available world map was downloaded from the website, presentationmagazine.com (<http://www.presentationmagazine.com/world-maps-vectoreditable-507.htm>). The map was created and edited in PowerPoint.

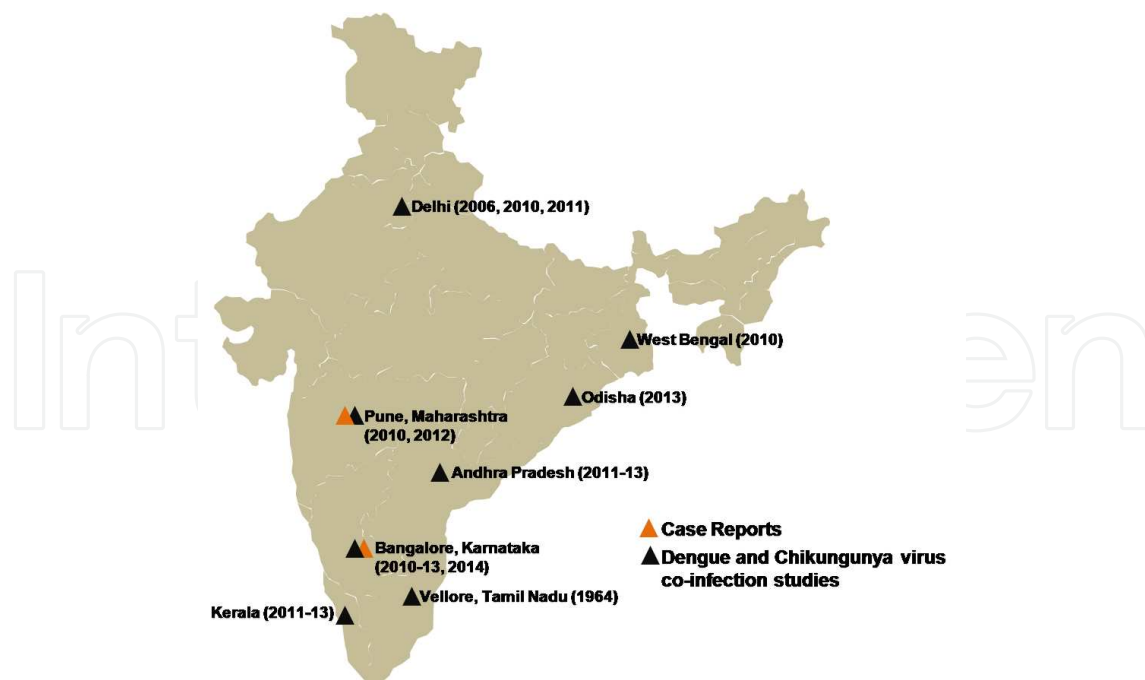


Figure 2. The map of India showing co-infection with Dengue and Chikungunya viruses. The map was downloaded from the website, presentationmagazine.com (<http://www.presentationmagazine.com/world-maps-vector-editable-507.htm>). The map was created and edited in PowerPoint.

Study site (Year)	No of samples tested	Method (DFA/culture/ ELISA/PCR)	No of Virus +ve (%)	Dengue virus +ve (%)	Chikungunya virus +ve (%)	Co-infections	Reference
Vellore, India (1964)	372	ELISA	206 (55)	11(3)	202 (54)	7 (2)	[14]
Delhi, India (2006)	69	RT-PCR	65 (94)	48 (70)	17 (25)	6 (9)	[4]
Delhi, India (2010)	432	RT-PCR	93 (22)	54 (13)	39 (9)	5 (1)	[15]
Delhi, India (2011)	87	RT-PCR	59 (68)	43 (49)	25 (29)	9 (10)	[16]
Odisha, India (2013)	204	ELISA	124 (56)	96 (47)	72 (32)	43 (19)	[17]
Kerala, Andhra Pradesh, India (2011-13)	1024	ELISA and RT-PCR	249 (24.3)	194 (18.9)	55 (5)	25 (2.4)	[18]
Bangalore, India (2010-13)	6554	ELISA	3824 (58)	3202 (49)	622 (10)	532 (8)	[19]
Pune, India (2010)	364	ELISA	175 (48)	121 (33)	79 (22)	25 (7)	[3]
West Bengal, India (2010)	550	ELISA	303 (55)	172 (31)	199 (36)	68 (12)	[21]
Gabon, Africa (2007)	773	RT-PCR	329 (43)	54 (7)	275 (36)	8 (1)	[8]
Gabon, Africa (2007)	4287	RT-PCR		367 (9)	1567 (37)	37 (0.9)	[5]
Madagascar, Africa (2006)	55	RT-PCR	38 (69)	24(44%)	4(7%)	10 (0.2)	[24]
Saint Martin, France (2013-14)	1502	RT-PCR and ELISA	635 (42)	65 (4)	570 (38)	16 (2.8)	[22]
Sri Lanka	54		41(76)	20 (37)	21(39)	3 (5.5)	[23]

Table 1. Studies on co-infections with Dengue and Chikungunya viruses

Chahar and colleagues conducted Dengue and Chikungunya virus specific RT-PCR on patients suspected of Dengue and/or Chikungunya fever in New Delhi, India [8]. Their study detected 6 (8.7%) co-infected patients. All 6 patients had fever, headache, joint pain, and low thrombocyte counts ($<100,000/\text{mm}^3$). Two of the co-infected patients had dengue hemorrhagic fever with central nervous system (CNS) involvement. In another study from Delhi, 5 (1.1%) co-infection cases were detected in the samples tested by RT-PCR [19]. All the co-infecting strains clustered with genotype II of Dengue 1 virus and ECSA genotype of the Chikungunya

virus on phylogenetic analysis. Concurrent infection with the Dengue and Chikungunya viruses were detected in 9 (10%) samples tested by RT-PCR in a recent study from our laboratory from New Delhi, India [20]. Genetic characterization of the co-infecting strains showed that CHIKV belonged to East Central South African genotype and Dengue strains belonged to the American African genotype of DENV-1. Co-infection with the two viruses was detected in 28 (13.7%) of the samples by RT-PCR in a recent study from Odisha, India [21]. Dayakar and co-workers [22] have recently reported co-infection by Dengue and Chikungunya viruses in 23% of the suspected patients by RT-PCR from Andhra Pradesh and 0.1 % from Kerala in Southern part of India. Sheikh and colleagues carried out IgM ELISA specific for Dengue and Chikungunya virus on samples collected from Karnataka, India [23]. Specific antibodies against both Dengue and Chikungunya virus were detected in 532 (8.1%) samples. Another investigation from Tirupati, a Southern part of India detected antibodies in 2 (2.7%) of the samples tested by IgM ELISA [24]. Gandhi and co-workers [7] have identified dual infection with Chikungunya and Dengue viruses in 25 (6.8%) of the patients by IgM ELISA from Pune. Dual infections with both the viruses were reported in 68 (12.4%) samples by IgM ELISA from West Bengal, an Eastern part of India [25].

Various investigations from different geographical regions have also described the co-infections with the two viruses (Figure 1), including 2.8% in France [26], 5.5% in Sri Lanka [27]. A serological study from Sri Lanka reported co-infection with the two viruses in 3 (5.5%) of the samples tested by IgM ELISA [27]. Various African countries have reported co-infection with the two viruses. Chikungunya and Dengue viruses caused a large simultaneous outbreak in southeast Gabon in Central Africa in 2010. Between 2007 and 2010, a total of 4287 acutely febrile patients were investigated for both the viral infections by quantitative real-time reverse-transcription polymerase reaction [9]. Out of the samples tested, 1567 were CHIKV-positive, 376 DENV-2-positive, and 37 (0.9%) co-infected. When a human case of co-infection was diagnosed in the above mentioned study, mosquitoes were captured around the patient's home during daytime. One CHIKV- and DENV-2-coinfected *A.albopictus* specimen was also detected in the study, which represented the first observation of dual mosquito infection in nature. Detection of co-infection of Dengue and Chikungunya virus in *A.albopictus* demonstrates the possibility that humans could be co-infected with the 2 viruses by the bite of a single mosquito. Viral loads were determined for the 24 co-infected, 121 CHIKV-positive and 52 DENV-2-positive patients. Mean values of Dengue and Chikungunya viral loads in co-infected patients were significantly lower than mean values in CHIKV- and DENV-2-mono infected patients. Thus, the investigators concluded that the two viruses exerted a suppression effect on each other. Phylogenetic analysis revealed that the CHIKV and DENV-2 isolates belonged to African clusters and they grouped together with strains reported from other parts of Africa. In another study from the same region, the samples were tested by real time RT-PCR and 8 (1%) were co-infections [12]. In another investigation conducted in Madagascar, another African country, dengue like cases were investigated by RT-PCR and ELISA. Ten (18%) samples were found to be co-infected by both Dengue and Chikungunya virus [28].

Simultaneous detection of the two viruses has been described in a number of case reports (Table 2) (Figure 1 and 2). A case report identified Dengue and Chikungunya viruses in an eight year old child at Mysore in Southern part of India [29]. Raut and colleagues recently

reported a case of multiple co-infections in a young man after his return from Nigeria [30]. The patient was positive for dengue xlink antigen as well as Dengue and Chikungunya virus by RT-PCR. Apart from Dengue and Chikungunya virus, the patient was also positive for the malarial parasite, *Plasmodium falciparum*. A recent investigation reported that a woman returning to Portugal from Angola was infected with Dengue 4 virus and ECSA genotype of the Chikungunya virus [31]. Another case of imported infection in patients who had returned to Taiwan from Singapore was reported [32]. Dengue and Chikungunya virus co-infection in this patient was confirmed by real time PCR and sero conversion for both the viruses in the convalescent-phase serum samples. The co-infecting viruses were identified as the Dengue virus serotype 2 and ECSA genotype of the Chikungunya virus. Dual infection with both the viruses was detected in a German traveler who was employed as a social worker in India [33]. Two different patients had infection with the two viruses in Malaysia [34]. An 80 year old patient from Sri Lanka was diagnosed with Dengue and Chikungunya virus infection by RT-PCR [35].

Study site (Year)	Age of patient (in Years)	Co-Infecting pathogens	Reference
Malaysia (2006)	Case 1: 28 Case 2: 22	DENV+CHIKV	[30]
Sri Lanka (2006)	70	DENV+CHIKV	[31]
Germany (2009)	25	DENV+CHIKV	[29]
Taiwan (2009)	12	DENV-2 +CHIKV	[28]
India (2012)	8	DENV+CHIKV	[25]
Portugal (2013)	50+	DENV-4+CHIKV	[27]
India (2014)	21	DENV+CHIKV+ <i>Plasmodium falciparum</i>	[26]

Table 2. Case studies involving Co-infections with Dengue and Chikungunya viruses

Additionally, the latest information of the ongoing outbreaks of these two arboviruses is available on the WHO/CDC/PAHO websites. The interactive map of recent and ongoing Chikungunya fever outbreaks is available at: <http://www.arcgis.com/apps/MapTools/index.html?appid=ce2372254ce743b79d332b43724cd9e5> and Dengue map, a CDC- health map collaboration is available at: <http://www.healthmap.org/dengue/en/>. These interactive maps report recent Dengue and Chikungunya outbreaks throughout the world and global risk areas. The map shows recent reports of local and imported cases based on official, newspapers and other media sources. Global risk areas are determined by consensus between sources including: national surveillance systems, published literature, questionnaires and formal and informal news reports.

Detection of high number of co-infection cases in some of the studies mentioned above shows that many Chikungunya cases go undiagnosed in Dengue endemic regions, thereby concealing the true burden of the Chikungunya viral infection. Further epidemiological and viral investigations involving larger patient group in the endemic areas are needed to define the precise role of Dengue and Chikungunya viruses in clinical presentation of these dual

infections. These studies will further assist to monitor the spread of these arboviruses and implementation of appropriate control strategies.

7. Dengue, Chikungunya and Zika virus co-infection in humans

Zika virus (ZIKV) is also an arbovirus and a member of Flaviviridae family. It was first identified in rhesus monkey in Zika forests of Uganda in 1947 [36]. The mode of transmission of Zika virus is same as for Dengue and Chikungunya viruses, i.e., through *Aedes spp.* The non-mosquito mediated transmission of ZIKV include sexual and mother to fetus transmission during pregnancy [37, 38]. The febrile illness due to Zika virus is very much overlapping with that of Chikungunya and Dengue virus Infections [39]. High grade fever, arthralgia, myalgia, retro-orbital pain etc are a few common symptoms. Subsequently, after a few sporadic cases of ZIKV in Africa, the first documented epidemic in Yap Island occurred in 2007 affecting more than half of the population in Yap Island [40]. Similarly outbreaks of ZIKV were reported from Pacific islands including French Polynesia, New Caledonia, Cook Islands, Easter islands, etc in 2013-14 that affected thousands of inhabitants [41]. In 2015 the largest epidemic of Zika virus started in Brazil that later spread to other American countries and Caribbean region affecting millions of people [42]. The Zika virus infection during ongoing pandemic is also found to be associated with many neurological complications like Guillain-Barré syndrome and microcephaly [43]. A recent report suggested that till 6 April 2016, 62 countries [44] (mostly in South and North America) have reported active transmission of ZIKV. Additionally, the virus has also been detected in returning travelers from 13 non-endemic countries [45-49].

Thus currently, the Zika virus is co-circulating with Chikungunya virus in many parts of world including Americas [36]. Recently in 2015 Villamil-Gomez and coworkers reported co-infection with Dengue, Chikungunya and Zika viruses in a 49 year old male patient from Columbia. The patient was reported with febrile illness with 38°C temperature, conjunctivitis, maculopopular rashes etc.. The blood sample collected from the patient was positive for Dengue and Chikungunya virus IgM antibodies. Additionally, the sample was positive by RT-PCR for ZIKV and DENV [50, 51]. The authors observed no synergistic effect of these infections in the patient [50]. Therefore, differential diagnosis of these arboviral infections should be done on patients during the ongoing ZIKV pandemic in this region for proper patient management. Subsequent detailed investigations will determine the affect of co-infections with these arboviruses on disease severity.

8. Conclusions

Dengue and Chikungunya viruses can cause dual infections in humans and in the mosquito vector. The significance of the Dengue and Chikungunya dual viral infections can be elucidated by measuring viral loads of each infecting virus and the effect of competitive suppression of the infecting viruses. Further investigations are needed on transmissibility studies in the mosquitoes using chimeric viruses. In addition, more elaborate clinical studies involving larger patient groups are required to ascertain the effect of severity of the disease in case of dual viral

infections. Also, the clinically suspected cases should be tested for both the pathogens in the endemic areas. This information is essential for early and timely diagnosis of the infecting pathogen and correlation of the clinical symptoms with mono or dual infections for appropriate patient management. It has been postulated that a recent increase in Dengue and Chikungunya virus co-infection may affect the evolution of these rapidly emerging viruses. In addition, the infectivity as well as the pathogenicity of these viruses may also be affected in future. Further, continuous surveillance for both Dengue and Chikungunya viruses is essential in the endemic areas for identification and characterization of these viral pathogens. This information will also help in the implementation of proper measures to control the outbreaks caused by these emerging viral pathogens.

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