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Short Communication

Molecular Diagnosis of *Chikungunya virus* (CHIKV) and *Dengue virus* (DENV) and its concomitant circulation in South Indian population

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ABSTRACT

Concomitant circulation of dengue and chikungunya viruses has been sporadically reported and needs to be individually diagnosed. Several sensitive molecular diagnostics are currently deployed for the diagnosis of both DENV and CHIKV, it is very difficult to delineate the two viral infections based on symptomology, as both share similar clinical presentation. Due to the overlapping nature of clinical signs in DENV and CHIKV infections, there is an urgent need for early and accurate diagnosis to avoid outbreaks aimed at initiating disease specific interventions. The study encompasses the diagnosis of clinically suspected 1024 DENV/CHIKV patient samples which were collected from AndhraPradesh (AP) and Kerala, India by using in-house developed RT-PCR (reverse transcription-polymerase chain reaction). The results show 43.8% DENV RNA infection and32% CHIKV RNA infection of the total samples obtained from AP. In contrast, samples from Kerala show dengue infection of 16.1%, CHIKV infection of 2.3%. In our study, we found that 23%, 0.1% of the samples were concomitant circulation for CHIKV/DENV in AP and Kerala respectively, suggesting the co-infection of these two viruses.

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1. Introduction

Having known for its menace in tropical countries such as Africa and Asia, dengue fever is caused by a class of pathogens called as *Flavivirus* that belongs to the family of Flaviviridae and Chikungunya, being caused by the *Alphavirus* of family *Togaviridae* (Buchen et al., 2010). Both diseases are transmitted to humans by day-biting *Aedes aegypti* and *Aedes albopictus* mosquitoes and almost cause similar clinical symptoms such as fever, rashes, joint pain; headache, fatigue, nausea, vomiting, and body pain are the hallmark for both the diseases that makes diagnosis difficult (Konstantin et al., 2007). Concomitant circulation of both these viruses in humans was reported and apprehended by serological analysis but their results did not pave clue about the on-going infection with both the viruses (Arankalle et al., 2007). Co-infection of these viruses presents similar clinical signs, but different disease patterns. Unavailability of viral specific diagnostic tools complicates medical management strategies and hence clinically viable and easily available molecular markers are the need of the hour. Here, a study was conducted to establish a handy diagnosis method for the screening of chikungunya and dengue fever through in-house designed RT-PCR approach.

2. Results

1024 patient blood samples from Kerala and Andhra Pradesh were tested for the screening of dengue and chikungunya. In the current cross-sectional study, we found that nearly 46/105 samples (43.8%) were positive for DENV, 34/105 samples (32%) were positive for CHIKV and 24/105 samples (23%) were positive for the presence of both viruses in the Andhra Pradesh region. In contrast to the above figures, patients from Kerala were found to be positive for dengue with 16.1% (148/919), positive for CHIKV at 2.3% (21/919) and surprisingly only 0.1% (1/919) of people were found to be carrying both the viruses. Serology analysis using dengue IgM in Andhra Pradesh and Kerala showed 60.9% (64/105) and18.8% (173/919), respectively (Table 3). To further validate our above findings, we ran RT-PCR to detect the presence of DENV RNA in blood samples of both Andhra Pradesh and Kerala patients. We concluded that, in Andhra Pradesh alone, 45.6% (21/46) of the samples showed positive for dengue serotype-2; 54.6% (25/46) showed positive for more than one dengue type such as dengue type 2 and 3; co-infection was detected in 39.1%; 0.6% (4/46) of the samples being positive for type 2 and 4; mere 6.5%of all patient samples showed the presence of D-1, D-1/2, D-1/4, D-1/3, D-3/4, D-1/3/4, D-2/3/4 serotypes (Table 4).

148/919 samples from Kerala were tested for DENV RNA by RT-PCR. Five samples could not be genotyped due to low copy number and 143 samples were analyzed for dengue serotype specific PCR. Among 143 samples, dengue serotype 2 occupied 35.6% (51/143) and the remaining 64.3% (92/143) of the samples were diagnosed with other subtypes of dengue such as D-4 of 16.7% (24/143), D-3 of13.2% (19/143), respectively. Co-infection of D-2 and D-4 was found to be 12.5% (18/143), D-2 and D-3 were 7.6% (11/143) and co-infection was 13.9% (20/143) of the samples tested (Table 4).

The BLAST data confirmed the amplified products to be that of DENV and CHIKV sequences. The phylogenetic analyses of the DENV sequences revealed that the amplified DENV samples clustered along with DENV-2, DENV-3 and DENV-4 genotypes (Fig. 2), while the representative sequenced CHIKV sample clustered with central African genotype (Fig. 3), results of RT-PCR showed statistical significant DENV alone

Table 1
Case definitions.

Case	Symptoms
Suspected	An acute illness characterized by sudden onset of fever with several of the following symptoms: joint pain, headache, backache, photophobia, arthralgia, rashes, etc.
Probable	Above symptoms with positive serology either when single serum sample was taken during acute onset phase or during the convalescence
Confirmed	A confirmation was done based on the following criteria: 1. 4-fold difference in HI antibody levels 2. Detection of IgM antibodies against *Chikungunya virus* 3. Virus isolation from plasma on cell cultures 4. Detection of CHIKV genomic RNA by RT-PCR