CHAPTER 1
INTRODUCTION

1.1. Background

There is an emergence and re-emergence of arboviruses such as dengue and chikungunya in India. Both diseases during the early phase of illness present as acute febrile undifferentiated illness affecting children and young adults. In a tropical country, like India, these diseases may mimic typhoid, leptospirosis and malaria. Currently, the methods used for detection of dengue include detection of NS1, IgM and IgG (Arya *et al.,* 2011).

Chikungunya virus (CHIKV) re-emerged after 32 years in Andhra Pradesh, India during late 2005. The first report of CHIKV in Kerala, India was documented in 2006 and more than 70,000 cases were reported from 14 districts (Sreekumar *et al*., 2010). During the re-emergence of CHIKV in Tamil Nadu nearly 1,00,000 people were affected in 2006, majority of patients were from Kanyakumari, Tirunelveli and Salem districts. A state in India has reported more than 10,000 cases of CHIKV especially in 2016 (National Vector Borne Disease Control Programme (NVBDCP)). A recent sero surveillance in different parts of Chennai revealed that 93% of participants were seropositive to DENV and 44% to CHIKV (Rubiano *et al*., 2015).

The Asian genotype of CHIKV was the predominant circulating type for several decades which was confirmed by molecular characterization of E1 gene (Arankalle *et al.*, 2006). However recently East Central South African (ECSA) has replaced the Asian genotype. E1 gene has been extensively studied and only limited data is available on the E2 region which is highly conserved and responsible for host cell interaction(Tsetsarkin *et al.*, 2009; Tsetsarkin & Weaver, 2011). Epidemic of 2005-2009 had severe clinical manifestations therefore we investigated E2 region in order to know the association of mutations and clinical manifestations. Mutations in the genotype of virus has conferred better vector adaptability, so that same mosquito can transmit both CHIKV and DENV (Coffey *et al.*, 2014). The clinical spectrum of CHIKV diseases has expanded which suggests that more target organs are affected (Her *et al.*, 2010). Whether this expansion is related to mutation is unclear. Epidemic of dengue are reported most during monsoon periods as it coincides with mosquito breeding. Most of the major cities are affected during this season.

Following the 2005-2009 outbreak of CHIKV, frequent reports of CHIKV were documented (NVBDCP). However, CHIKV is often left uninvestigated and remains a clinical diagnosis. However, the excruciating arthralgia and persistent arthralgia/arthritis for several months necessitates therapy. Elevation of certain cytokines in CHIKV patients indicates the role of cytokines in causing arthralgia.

In 1996, first major DHF outbreak was caused by DENV-2, another DF outbreak was caused by DENV-3 almost after a decade (2003). Since now dengue has now become endemic in India, all the four serotypes of DENV are found to be in circulation as well as co-circulation. However, predominant serotype is not the same every year (Gupta *et al.*, 2014).

Molecular techniques such as real time RT-PCR have a significant role in assessment of viral load and for serotyping of dengue. Assessment of viral load will help to determine the progression of the disease. High viral load indicates rapid replication of the virus and it may lead to severe disease as it induces cytokine storm (Martina *et al.*, 2009). Real-time RT-PCR techniques and in-house RT-PCR have been developed for detection of DENV by targeting CPrM or NS1, and for CHIKV by targeting E1 and E2 (Edwards et al., 2007) but are underutilised. Severe manifestations of dengue demand an early diagnosis.

Development of dengue haemorrhagic fever (DHF)/dengue shock syndrome (DSS) in dengue patients is also due to cytokine elevation. This leads to hepatic and renal involvement. Involvement of human cytotoxic factor (hCF) mediates immunopathogenesis of dengue which leads to shifting of Th1 response to a Th2 response resulting severe dengue (DHF). Cytokines play a significant role in the progression of mild disease to severe dengue and causing damage

to endothelial cells of capillaries, resulting in fluid leakage which leads to DSS/DHF (Chaturvedi et al., 2001). Multiple strategies are needed to detect and control the epidemic of dengue.

Though different assays are available for detection of CHIKV and DENV such as ELISA for detection of antigen, IgM and IgG antibodies, RT-PCR and Real-time PCR, they have their own limitations. Determination of cytokine profiles in acute phase of illness will help to assess the disease progression.

Several studies have been performed on cytokine profiling of dengue (DEN) and chikungunya (CHIK) patients, none of the studies have correlated the cytokine profile between these both viral diseases. Cytokine profile was determined in the viremic and non viremic phase of both DEN and CHIIK patients to determine the significance of cytokine elevation during presence/absence of virus. Among DEN group cytokine profile was also determined in the convalescent phase to know about the cytokines responsible for the recovery of the disease. Patients were grouped as probable dengue, dengue warning signs and severe dengue based on 2009 WHO classification in order to know the cytokine involved in each group.

1.2. Need for the Study

1.2.1. Development and evaluation of Multiplex PCR for Chikungunya and Dengue

The clinical features of Chikungunya are similar to those seen in dengue virus infection and the areas of occurrence of CHIKV overlap with those endemic for Dengue fever (DF) and DHF. Thus, the simultaneous diagnosis of two infections is essential for clinical management and epidemiological study in the tropics. The aim of this method is to develop an In-house Multiplex RT-PCR for the simultaneous identification of Chikungunya and Dengue viruses. Evaluation of the assay will be done with comparing RT-PCR for CHIKV and DENV.

1.2.2. Phylogenetic characterization of Chikungunya viruses prevalent during outbreak

The epidemiology and clinical features of chikungunya have a number of similarities to those of dengue viruses. Both the viruses are prevalent in the Indian subcontinent. This is to determine the phylogeny of CHIKV isolates, isolated during recent outbreaks in Tamil Nadu and Kerala, by PCR-Sequencing of the partial sequences of envelope gene E2. This study will render the prevalent CHIKV genotypes/clades in this region to be identified. Further determining the circulating genotypes and its associated large scale outbreaks will help in understanding the spread of CHIKV infection and its control measures.

1.2.3. To determine the cytokine profiles between Dengue and CHIKV infected persons and persons who have cleared infection

Measurement of the cytokine profiles from patient samples was performed by using standard cytokine specific ELISA to identify the profile of cytokines during CHIKV and DENV infections. These indicate that the cytokines determine the viremic status. Since Dengue viral infection is very similar to chikungunya infection, determining the cytokine profile of dengue viral infections will help to examine whether these agents elicit similar cytokine responses.

1.3. Scope of the Work

CHIKV genome consists of NSP1, NSP2, NSP3, NSP4, C, E1, E2 and E3. Most of the molecular characterization study was done based on E1 region. E2 region was selected for molecular characterization since it is highly conserved region(Li *et al*., 2010, Voss *et al.*, 2010). The clinical features of Chikungunya are similar to those seen in dengue virus infection and the areas of occurrence of CHIKV overlap with those endemic for Dengue fever (DF) and dengue haemorrhagic fever (DHF). Performing two separate RT-PCR is expensive and time consuming(Mishra, *et al.*, 2011). Therefore, multiplex RT-PCR was developed for the simultaneous detection of CHIKV and dengue. Though there is data available related to cytokine profiles of chikungunya and dengue, there are no reports available with the comparison of cytokine profiles of chikungunya and dengue in the same study.

1.4. Aims and Objectives:

Broad aim of the study is to determine the Molecular and phylogenetic characterization of Chikungunya (CHIKV) and molecular detection of Dengue virus. To study the Immunological response of human host system to Chikungunya infections and Dengue infections

1.4.1. To develop and standardize an in-house Multiplex RT-PCR for the simultaneous identification of Chikungunya and Dengue viruses. To analyse the sensitivity and specificity of Multiplex RT-PCR for detection of CHIKV and Dengue and to compare with conventional RT-PCR method in the diagnosis of these viral infections.

1.4.2. To determine the phylogeny of CHIKV positives during the outbreak by sequencing the partial sequences of envelope gene E2. To identify the prevalence of CHIKV genotypes/clades in this region.

1.4.3. To determine cytokine profiles between Dengue and CHIKV infected persons and persons who have cleared infection. To correlate cytokine profile between Dengue and CHIKV viral infection.

1.5. Research Questions

In order to achieve the study objectives, the following research questions were formulated:

1. Can RT-PCR be useful in the detection of causative agents during an outbreak in resource limited settings?
2. What is the performance of multiplex RT-PCR in the detection of DENV and CHIKV?
3. What are the evolutionary changes in CHIKV E2 region and does this relate to disease severity?
4. Which are the CHIKV genotypes/clades in this region?
5. How are the cytokine profile pattern with DENV and CHIKV?
6. Are the cytokine profiles in DENV and CHIKV similar or different?
7. What are the characteristic features of cytokine in DENV and CHIKV disease severity?