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| **Title of the Thesis:** | **Molecular studies of Chikungunya virus isolates from different parts of India** |

# ABSTRACT

Chikungunya (CHIK), viral disease caused by arthropod borne Chikungunya virus (CHIKV) and transmitted by *Aedes* mosquitoes. CHIKV re-emerged in 2005 with unprecedented magnitude in Indian Ocean Islands and South East Asia. Several large and explosive outbreaks of chikungunya infection in the past decade jeopardized the public health professionals and stimulated renewed interest in CHIKV. Indigenous to tropical world, CHIKV is expanding its geographical horizons towards temperate areas of world. After re-emergence, estimated 1 million symptomatic cases and 0.1% fatality case reported per year globally poses epidemiological burden and productivity loss to the community.

After the quiescence of about three decades, CHIKV re-emerged in India during 2006 and gave rise to outbreaks in different geographical locations by affecting millions of people. CHIKV accumulated biologically important mutations in the absence of herd immunity, lack of vector control and globalization of trade and travel facilitated the recently changed epidemiology. The molecular typing of chikungunya viruses during early phase of the outbreak may be useful in the absence of any specific vaccine or antiviral therapy by providing substantial warning signal to health authorities about the virulent or mild genotype of chikungunya virus circulating in the area. Hence, molecular epidemiologic study is necessary for keeping a close vigil on the changing pattern of CHIKV genomic heterogeneity, monitoring emergence of mutants strains and tracing the origin and source of infection/ route(s) of transmission. The present study was undertaken with these objectives:

- Molecular identification of chikungunya virus by using different combination of *nsp1, E1, E2* gene regions.
- Genetic heterogeneity, sequence diversity and phylogenetic analysis of chikungunya virus isolates from different geographic regions of India.
- Relevant cytokines analysis for chikungunya virus.

The present study encompasses the following findings:

- Studies on the suitability of different genes of CHIKV indicated that molecular diagnosis based on 'single-gene' may miss detection in some otherwise positive cases.
- RT-PCR using combinational approach of different gene regions could detect CHIKV in nearly 100% of the positive samples.
- It is therefore, appropriate to carry out RT-PCR in at least two gene regions (if not more, preferably combination of *nsp1* and *E2* gene regions) in order to diagnose all CHIKV infection cases.

- *E1* gene region gave more elaborate information about molecular epidemiological aspects of CHIKV compared to other sequenced *nsP1* or *E2* gene regions.
- The genetic sequence data obtained from this study revealed possible origin or route(s) of transmission of the CHIKV in different geographical areas of India.
- Genomic analyses of the strains of 2006-2011 of CHIKV circulating in different zones of India revealed that all new strains of Indian CHIKV from South, Central, West, Eastern and North part of India closely related to the circulating Reunion-06 strain, indicating their possible origin from Indian Ocean Islands.
- Phylogenetic tree revealed that all studied chikungunya strains along with Reunion-06 strains grouped with East Central South African genotype. The finding indicates active circulation of ECSA genotype throughout India by replacing erstwhile Asian genotype.
- Currently circulating strain of CHIKV in North India had its origin from '2006 epidemic strain of South India' that moved towards North India via Western Central parts between 2006-2010 in a phased manner with dominance of East Central South African genotype.
- Chikungunya virus emerged in Delhi during 2006 and existed till 2009. Later, CHIKV gave rise to its first outbreak co-dominantly with dengue virus in 2010. Even in the year 2011, sporadic cases of chikungunya virus infection were also reported.
- From north, western and eastern India, all sequenced samples showed unique amino acid replacement lysine (K) by glutamic acid (E) at position 211 in *E1* gene (K211E) of East Central South African genotype of chikungunya virus. This amino acid K211E is highly conserved in CHIKV strains of the Asian genotype whose natural vector is *Aedes aegypti*.
- Two sub-lineages of East Central South African genotype of CHIKV was found to be circulated in India parallel to abundance of *Aedes aegypti* and *Aedes albopictus* having K211E and A226V mutation respectively.
- Entropy plot analysis and phylogenetic analysis demonstrated the similar results.
- The strong positive correlation was found between chikungunya virus infection during acute phase and cytokines induction.
- The levels of inflammatory cytokines IL-1β, IL-6, IL-8, and IL-10 were found to be elevated in acute phase infection of chikungunya virus while the levels of IFN-γ and TNF-α remained stable. Increased level of biomarkers (IL-1β, IL-6, IL-8, and IL-10) seemed to be associated with severity of the disease during acute phase.

The present work acquires great significance, as it emphasizes the importance of establishing rapid and accurate diagnosis of CHIKV infection and their typing, which is an extremely important pathogen of recent times. The results from the present study provide molecular epidemiological pattern of re-emerged (2006-2011) CHIKV, indicated active circulation of single ECSA genotype throughout India that provides important basis for vector control programme and the disease containment. Functional studies on reported genetic variations or mutations would help in understanding correlation of CHIKV virulence and pathogenesis. Genetic variability data of studied Indian strains provide basis for developing effective vaccine for our population. With the help of studied cytokines levels during acute phase CHIKV infection, mild and severe form of disease can be distinguished. Cytokine levels may act as indirect predicator(s) and may also be responsible for sequelae of the disease. The findings of specific immune mediators involved in disease progression gives a better idea of possible therapeutic interventions.