Chapter - 5

SUMMARY AND CONCLUSION

Based on clinical features, Chikungunya fever was suspected among patients in Kadapa district of Andhra Pradesh. Fever (99.2%), arthralgia (97.7%), myalgia (89.1%), difficulty in walking (78.1%), joint stiffness(77.3%), morning stiffness (76.6%), sleeping disturbances (75.8%) and headache (70.3%) were the major symptoms observed in >70 % of the patients. Multiple joints were affected and 78.9% of the patients suffered from severe pain. Rashes, retro-orbital pain, cough, running nose, oral ulcers, weight gain and diarrhoea were reported in <20 percent of the patients. Weight gain (3.9%), rashes (5.5%) and diarrhoea (5.5%) were the lowest recorded symptoms among CHIKV infected patients.

Rapid Immuno Chromatographic Assay (RICA) was found to be more sensitive than IgM Antibody Capture Enzyme Linked Immuno Sorbant Assay (MAC-ELISA) for the early diagnosis of Chikungunya that helps doctors in the line of treatment. A Reverse Transcriptase - Polymerase Chain Reaction (RT-PCR) based diagnostic assay was developed which could amplify a 330bp product specific to CHIK E1 gene. The 330bp PCR products were cloned and the resulting recombinant plasmids (pGEM-CHIK/KDP UHC and pGEM- CHIK/Devapatla) were transformed into *E. coli* DH5α cells. The recombinant clones were confirmed, sequenced and deposited in the GenBank (KF587904 and KF587905). The recombinant plasmid pGEM-CHIK/KDP UHC was subsequently used as a positive control for screening of CHIKV suspected samples during the outbreaks in Andhra Pradesh. Out of 68 samples screened, 20 were positive for CHIKV specific RNA by RT-PCR assay. The sequence obtained from RT-PCR assay were subsequently used for phylogenetic analysis of the CHIKV isolates. Thus the RTPCR assay also served as a tool for rapid clustering of isolates

and for the confirmation of CHIKV from different outbreaks. Phylogenetic analysis of the CHIK/KDP UHC and CHIK/Devapatla isolates was studied with the other isolates at nucleotide level and amino acid level.

In conclusion, we performed the CHIK IgM antibody based early diagnosis of Chikungunya using RICA and MAC-ELISA which found to be easy to perform and cost effective and to be useful for the field diagnosis of the disease. We compared the sensitivity of both assays and found that RICA was more sensitive than MAC-ELISA for the early diagnosis of the disease which helps the doctors in the line of treatment. We also developed a rapid and sensitive RT-PCR diagnostic assay for the detection of CHIKV at molecular level. The RT-PCR assay will be applicable to other CHIKV epidemics, especially in the Indian subcontinent, where an extensive outbreak is ongoing and will serve as a rapid diagnostic assay during CHIKV suspected outbreaks/interepidemic periods. The CHIKV outbreak in Kadapa district of Andhra Pradesh was severe and the impact on human health generally long lasting particularly with reference to prolonged arthralgia and oedema. Asymptomatic cases were encountered during the present study. From the sequence comparision and phylogenetic analysis of the present study isolates with other reported isolates we confirmed that these isolates were Chikungunya viruses circulating in Kadapa district of Andhra Pradesh.