

Investigation of Dengue Virus Serotypes and Genetic Variability in Relation to Disease Severity and Transmission

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ABSTRACT

Sri Lanka has been experiencing more or less periodic dengue outbreaks. **Methodology:** Acute phase blood samples were collected from patients clinically suspected of having dengue as determined by the WHO criteria (WHO 1997) within 4 days of onset of fever, from the hospitals in the Sri Lankan districts of Colombo, Gampaha, Kandy, Kurunegala throughout the year. Dengue vector *Aedes* mosquitoes were collected from the above geographical areas where positive cases were reported. Pooled mosquitoes and sera were subjected to analysis. RT-PCR and RT-PCR-LH established methods were used to detect dengue virus. Envelope glycoprotein gene (E gene) of DENV serotypes were sequenced and were compared with available sequences representative of the Americas, Oceania, Africa, South Asia, East Asia, and the Middle East. Deduced amino acid sequences of the Present Study Sri Lankan isolates (PSSL-iso) were compared with the past Sri Lankan isolates and South Asian isolates. Sequences of DF and DHF patients were compared as well. Clinical symptoms among serotypes in relation to severity were investigated.

Results and Discussion: In each district a minimum of 3 serotypes were in circulation. DEN-2 and DEN-3 were the most abundant. There was greater degree of conservation among the geographical isolates of DEN-2 compared to that of DEN-3. Evolutionary pressures act on the two serotypes differently despite both being co-transmitted within close proximities. Specific substitutions or substitution patterns which could differentiate between DF and DHF/DSS were not found. However phylogenetic analysis in DEN-3 revealed clustering of five isolates which showed an increased tendency to be associated with DHF or severe form of disease than the rest which harboured amino acid substitution Asn₃₈₃→Lys₃₈₃ on the lateral ridge of ED-III domain within the “informative sites” or sites known as unique conserved site for G-III. DEN-2 isolates belonged to the Cosmopolitan genotype were shown to have more of an Indian ancestry than a Sri Lankan one as they were closer in terms of phylogeny to the Indian origin isolates than to the previously existed Sri Lankan isolates. DEN-2 harboured unique Ile₂₂₆ lying within the Previously Determined Antigenic Regions (PDAR) a substitution which was not present in any of the South Asian isolates analysed. Simultaneous co-infections by DEN-2 & DEN-3 and DEN-4 & DEN-3 surprisingly lead to a reduction in disease severity. The results indicated that it is not in fact the number of amino acid substitutions which could determine the severity of disease but the type of substitution and the position within the domains which they occur. Most abundant vector species found in all districts was *Aedes albopictus* and all pools positive for DENV were *Aedes albopictus* pools. Moreover positive DENV transmission was observed in localities where Breteau index (BI) was as less as 5.55 suggesting the importance of the role of *A. albopictus* as an “underrated vector”. The findings in total suggest that all districts studied are hyperendemic to dengue and Sri Lanka is highly susceptible to successive outbreaks in the future while the difference of evolutionary pressures acting on the predominant serotypes and the reasons said above are likely to shape the nature of future outbreaks

Abbreviations not given in the text above : Reverse Transcription- Polymerase Chain Reaction (RT-PCR), Reverse Transcription- Polymerase Chain Reaction Liquid hybridization (RT-PCR-LH), Dengue virus (DENV), DENV serotype-2 (DEN2) DENV serotype-3 (DEN3), DENV Genotype-III (G-III), Envelope glycoprotein ectodomain III (ED-III)