

Cellular and virological changes with reference to cytopathic effect and virus load in dengue virus co-infections and super-infections *in vitro*

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Dengue virus (DENV) infection is caused by different DENV types: DENV-1, DENV-2, DENV-3 and DENV-4 depending on the prevalence of these types in a particular locale. Since 1960, all four DENV serotypes have been circulating in Sri Lanka and now these DENV types are hyper-endemic. Risk of co- and super-infection is high in a country like Sri Lanka due to the hyper-endemicity of these viruses. There have been a few case reports on DENV co-infections in patients in Sri Lanka. However, experimental studies on co- and super-infections are scanty to understand the virological and cellular changes in co- or super-infections with different DENV serotypes.

Hence, this study was conducted to understand the virological (virus load) and cellular changes (cytopathic effect - CPE) in experimental co- and super-infections with different DENV serotypes in Vero cells. Each DENV serotype was used to infect the Vero cells and incubated for 96 hrs. Cells were observed using the inverted microscope for CPE every 24 hours. After 96 hours of incubation, different DENV was/were harvested along with the Vero cells and the viral RNA was extracted using a validated RNA extraction system (Qiagen, Hilden, Germany). The DENV RNA samples were then subjected to quantitative reverse transcription polymerase chain reaction (qRT-PCR) to determine the viral loads in different infections used for the experiment.

The current study shows that the DENV-2 is an aggressive CPE producer. DENV-1 causes less CPE in Vero cells when compared to DENV-3. DENV-4 does not cause much CPE in Vero cells. DENV-2 has a higher ability to co-infect with other DENV serotypes and it can produce a high number of progeny comparing to other DENV types. DENV-3 takes a longer time to establish and thrive in the environment, whereas DENV-1 and DENV-4 are equally competitive and thrive in the environment depending on which serotype infects first. Also the current study findings suggest that a particular DENV infection needs to be present over a period to increase its progeny. When a DENV serotype establishes itself in the experimental environment, the next infecting serotype has to exert pressure to initiate replication.

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