ABSTRACT

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Molecular and serological analysis of dengue virus (DV) isolates and investigation of DV-mediated pathogenesis towards identifying DV antivirals.

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Dengue virus is a member of the family *Flaviviridae*, genus Flavivirus, and is transmitted to humans by the bite of female *Aedes* mosquitoes. Serum samples from dengue fever patients were collected during 2017-18 and were passaged in Vero cells. Then nucleic acid level screening was done and representatives of three serotypes (DV1, 2 & 3) were isolated. DV type 2 was most prevalent as has been found in this study. qRT-PCR was done to determine the virus titer and to select suitable ones for stock preparation. The selected samples were passaged three times in mosquito cell line C6/36 (*Aedes albopictus* larvae cells). Followed by virus titer was determined and stocks of four clinical DV isolates were standardized.

Hepatic dysfunction is an important feature of dengue as evident from various clinical reports, but the direct effect of dengue virus clinical strains on liver cells is not yet clear. So, the effect of clinical dengue virus and non-structural protein-1 (NS1) on human liver cell line (Huh7) was investigated. Dengue virus NS1 is one of the main players of pathogenesis and immune modulation. To study its effect, NS1 of three serotypes (DV-1,2,3) were cloned in pcDNA3.1(+) vector.

We observed that both DV infection and NS1 expression can induce apoptosis in liver cell lines as observed from cleaved caspase 3 expression. Only NS1 expression was enough to induce apoptosis. Now, to access the effect of NS1 in comparison with whole DV, we standardized the infection and NS1 transfection in such a way that similar amounts of NS1 were secreted in both the cases. Under such conditions, cellular apoptosis was further evaluated using apoptotic DNA ladder assay and TUNEL assay. Surprisingly, DV infected cells were with very less apoptotic DNA breaks in comparison with NS1 transfected cells, although both were expressing cleaved caspase 3. So, it appeared that DV is slowing down the apoptosis which is an antiviral response. To understand this phenomenon, DV infected cells were treated with Camptothecin (chemical inducer of apoptosis, Topoisomerase I inhibitor) and apoptotic DNA pattern was analysed. It was observed that apoptotic DNA is much less in infected cells in comparison with only Camptothecin treatment. This shows that DV protects cellular DNA as a strategy to delay the apoptosis, so that the window for virus replication and progeny formation can be extended.

As NS1 was found to be a potent viral protein for pathogenesis, the expression and secretion pattern was studied in detail for a target to intervene the secretion of NS1. NS1 expresses in ER; then travels to plasma membrane (PM) through trans-Golgi network, forming dimer. Then three dimers assemble on PM to form soluble hexamar with a lipid core. We designed peptides to bind with the hydrophobic β-sheet platform of NS1 dimer with a view to prevent hexamer formation. We tested the peptides on DV infected cells but there was no statistically significant reduction in NS1 secretion.

In early days of COVID19, there was a report that two SARS-CoV-2 infected patients' serum samples came positive in dengue IgG and IgM strip tests. This raised the possibility of false positive results. So, we tested the opposite scenario i.e. effect of DV serum on SARS-CoV-2 strip tests. In order to confirm the absence of SARS-CoV-2 antibodies (Abs), we used DV serum samples which were archived in 2017. It was found that 5 of 13 samples were positive in SARS-CoV-2 Abs lateral flow-based strip tests. Now, in dengue endemic countries such as in India, 48% population is seropositive for dengue which can result in many false positive results in rapid SARS-CoV-2 Abs tests. This was the first report that dengue serum samples can cross-react with SARS-CoV-2 antigen. We further tested COVID-19 serum from highly dengue endemic region (Kolkata) in DV rapid Abs tests and ELISAs and found very high cross-reactivity as well. Further, the effect of SARS-CoV-2 Abs on DV infectivity was tested by means of virus neutralization (VNT) assay. COVID-19 serum samples, including those with no previous dengue history neutralized clinical DV. So, SARS-CoV-2 Abs tests are needed to be supplemented with other tests such as nucleic acid tests for proper diagnosis, especially in areas where both the viruses are co-endemic now.

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