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Immunoinformatic Design of a Multi-Epitope Vaccine Targeting Co-Circulating Dengue and Zika Viruses in Sri Lanka and South-East Asia

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Dengue virus (DENV) and Zika virus (ZIKV) are mosquito-borne flaviviruses threatening public health mainly in Sri Lanka and South-East Asia. Their co-circulation, coupled with limitations of existing vaccines and the risk of Antibody-Dependent Enhancement (ADE), necessitates safer and broadly protective vaccine strategies. This study employed comparative genomics and immunoinformatic approaches to identify conserved immunogenic targets and design a Multi-Epitope Vaccine (MEV) against all four DENV serotypes and ZIKV. 1,928 high-quality, human-derived DENV and ZIKV polyprotein sequences from seventeen countries were retrieved from the NCBI Virus database following stringent quality control. Multiple Sequence Alignment using Fast Fourier Transform (MAFFT version 7) identified conserved regions across viral genomes, which were evaluated for antigenicity, allergenicity, toxicity, and immunogenic potential. B-cell, cytotoxic T lymphocyte (CTL), and helper T lymphocyte (HTL) epitopes were predicted and screened using immunoinformatic tools, and population coverage analysis assessed the global applicability of selected epitopes. Sequence analysis revealed 207 highly conserved regions among DENV serotypes and 51 shared with ZIKV, predominantly within the NS3 and NS5 proteins. Screening identified 27 B-cell, 15 high-confidence CTL, and 239 HTL epitopes with immunogenic potential, and the combined CTL–HTL set achieved an estimated 99.99% global population coverage. Five vaccine constructs (MEV1–MEV5) were designed using selected epitopes, linker sequences, and molecular adjuvants, and all were predicted to be antigenic, non-allergenic, and non-toxic, with favorable safety and immunogenicity profiles. *In silico* evaluation of physicochemical, structural, and immunostimulatory properties identified MEV4 and MEV5 as the most promising candidates, with superior stability and predicted immunogenic performance. These conserved epitopes enabled the rational design of a MEV with potential cross-protective efficacy against both co-circulating flaviviruses. These findings provide a foundation for developing broadly protective flavivirus vaccines. However, the immunogenicity, safety, and protective efficacy of the constructs require further validation through *in vitro* and *in vivo* studies.

Keywords: Dengue virus, Zika virus, multi-epitope vaccine, reverse vaccinology, immunoinformatics

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