Studies on biomolecules associated with vascular dysfunction in Dengue Haemorrhagic Fever / Dengue Shock Syndrome

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Abstract

Dengue fever is a rapidly emerging tropical disease and an important cause of morbidity in its severe form worldwide. This fever remains a global health concern, causing >500,000 hospitalisations annually due to severe dengue; only in Southeast Asia, the Pacific and the Americas. This self-limiting, acute febrile illness is caused by the dengue virus and is transmitted through Aedes mosquitoes. Most dengue infections result in mild symptoms but a subset of cases progresses to severe forms, known as severe dengue [Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS)]. A wide spectrum of the pathophysiology is associated with the transition of dengue fever to severe dengue, which is driven by the host immune response.

In this doctoral study, a total of 620 samples were included. Among them 380 samples were from dengue-suspected hospital-admitted patients and 240 samples were from the early phase of dengue fever for molecular serotyping. Dengue samples were screened for the presence of dengue NS1 antigen and IgM/IgG antibodies by enzyme-linked immunosorbent assay for dengue early phase and late phase identification. From early-phase samples, viral RNA was extracted from NS1 seropositive samples and subjected to molecular serotyping, genotyping and phylogenetic analysis. Co-circulation of all four dengue serotypes with DENV-2 as the prevalent serotype in the year 2018 (76%) and 2019 (47%), whereas DENV-4 in the year 2020 (47%), DENV-3 in 2021 (72%) and 2022 (55%) were observed. Phylogenetic analysis of prevalent serotypes showed Genotype-I of DENV-4 and Genotype-III of DENV-3 whereas Genotype II of DENV-2 was the major circulating DENV strain during the study period. Dengue fever-associated clinical manifestations, biochemical parameters and liver functional profile observed among hospital-admitted dengue patients. A multivariate logistic regression approach was used for making a regression model including dengue-associated clinical symptoms. 70% of patients showed thrombocytopenia, with petechia being the most common bleeding manifestation. A significant change in trends of dengue-associated clinical manifestations and differential expression of liver functional profile with different phases of transition of dengue fever was observed in this study population.

A mass-spectrometry-based proteomic approach was used to find the candidate proteins biomolecules associated with the pathogenesis of a severe form of dengue fever. We uniquely performed this prospective study among hospital-admitted dengue-infected patients from different phases (acute and critical) of dengue fever at two time points. Pairwise patient samples were subjected to high-throughput qualitative, quantitative proteomic, protein array and bioinformatics analysis to find the novel biomolecules and elucidate their intricate molecular networks underlying the pathophysiology of severe dengue. Pathway analysis was performed using PANTHER, Reactome and KEGG databases to find significantly enriched pathways and proteins. Protein-Protein Interaction (PPI) network between the dengue virus and host proteins was depicted in the search for

proteins associated with severe dengue pathophysiology. The set of elucidated proteins was validated via Western blot, Real-Time PCR and ELISA techniques. This study has validated expression patterns of Apo AI, AII, AIV, ApoB and ApoE among the apolipoproteins and E-Cadherin (epithelial cadherin), VEGF (Vascular endothelial growth factor), FGFR1 (fibroblast growth factor receptor 1), VCAM1 (Vascular cell adhesion molecule 1), IRF3 (Interferon regulatory factor 3), IFN-γ (Interferon-gamma) and ANGPT1 (Angiopoietin 1) among the endothelial proteins and cytokines from the significant proteins list via Western blot. Ten candidate genes were selected for Real-Time PCR-based validation. After narrowing down the Real-Time PCR result, group of top six biomolecules i.e. PTX3 (Pentraxin 3), LBP (Lipopolysaccharide Binding Protein), Fibronectin, IGFBP-2 (Insulin-like Growth Factor Binding Protein-2), POST (periostin) and Serpin were validated as top-notch proteins associated with severe dengue pathophysiology via ELISA-based mass validation from hospital-admitted patient's samples. The ROC curve's logistic regression analysis of signature proteins identified via ELISA has an average empirical AUC of above 0.7 with 95% CI. Protein docking results reveal a significant interaction between viral and host proteins, which is linked to severe dengue pathophysiology. STRING analysis depicted the protein-protein interactions of candidate proteins.

In conclusion, a comprehensive understanding of severe dengue pathogenesis requires a multidimensional approach that encompasses dengue virus changing patterns in a population, viral-host interactions, immune response dynamics and vascular dysfunction mediated by protein biomolecules. The identified eminent biomolecules panel and clinical markers from the liver have the potential to enhance predictive and diagnostic accuracy, risk stratification, patient management and therapeutic interventions among severe dengue patients.

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