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Title: Development of Point-of-Care Immunoassay System for rapid Diagnosis of Severe Dengue

Abstract

Background: Dengue an arboviral infection, has recorded a dramatic increase in prevalence in the past few decades. The majority of these cases are asymptomatic, with a minor fraction of them resulting in mortality. Current assays cannot predict the severity of the infection due to a lack of identifiable associated protein biomarkers. The absence of specific protein biomarkers significantly hampers the early identification of individuals at high risk of developing severe dengue. This can therefore result in inadequate or delayed interventions, which could worsen the disease's progression and raise the chance of death. By focusing early intervention efforts on high-risk people, healthcare providers may be able to lower the prevalence of severe dengue fever and enhance patient outcomes.

Additionally, the availability and promptness of dengue diagnosis and severity evaluation would be greatly improved by the founding of point-of-care diagnostic tools that incorporate these novel biomarkers. These tests could be used in distant or resource-constrained environments, where prompt and precise diagnosis is essential for efficient disease management.

Aim: This research focuses on the identification, validation, and utilization of protein biomarkers associated with dengue severity. This endeavor involves applying advanced proteomic techniques to comprehensively analyze the protein profiles of dengue patients with severe and nonsevere illnesses. By comparing the proteomic mode of severe and non-severe cases, we aim to uncover differentially expressed proteins that could serve as potential biomarkers for predicting disease progression. The ultimate goal of this research is to apply these identified and validated biomarkers to develop a lateral flow immunoassay (LFIA) for the rapid and accurate assessment of dengue severity. LFIA technology offers several advantages, including its ease of use and cost-effectiveness, making it suitable for point-of-care (PoC) applications. By incorporating the identified biomarkers into LFIA strips, we aim to create a diagnostic tool that can provide clinicians with a rapid and reliable assessment of dengue severity, causing timely and appropriate management decisions.

Methodology: To identify potential biomarkers associated with dengue severity, we employed LC-MS/MS-based proteomic analysis on the protein profiles of patients with severe and non-severe dengue infections. The identified biomarkers were subsequently validated using Western blotting and ELISA. Building upon these findings, we developed a lateral-flow-immunoassay (LFIA) incorporating the validated biomarkers. The newly developed assay was then evaluated using confirmed dengue samples to determine its sensitivity, specificity, positive predictive value, and negative predictive value. The newly developed assay could conclusively identify severe cases of infection.

Result: Proteomics analysis revealed 144 up-regulated and 89 down-regulated proteins between severe and nonsevere dengue cases. MASP-1, VTN, and TSP-1 were selected for this study due to their roles in the complement system and coagulation cascade, respectively. These pathways are known to be dysregulated in severe dengue, suggesting their potential involvement in disease pathogenesis. This research found that the levels of MASP-1 (Mannan Associated Serine Protease-1), TSP-1(Thrombospondin-1), and VTN (Vitronectin) were significantly altered in patients with severe dengue compared to those with non-severe infections. The combination of TSP-1 and platelet counts was evaluated for their predictive performance which showed almost 100% sensitivity and specificity and 1.000 for AUC (Area Under Curve). Using lateral flow immunoassay (LFIA), these biomarkers (MASP-1 and VTN) could effectively differentiate between severe and mild cases of dengue. This suggests that a combination of TSP-1 with platelet and LFIA-based testing of MASP-1 and Lectin-based LFIA with VTN could be a valuable tool for early identification of patients at risk of severe dengue and inform timely clinical management.

Conclusion: This study leveraged proteomics to conduct a comprehensive analysis of proteins on a large scale. A significant breakthrough was achieved by combining TSP-1 with platelet counts using CombiROC and developing a point-of-care immunoassay utilizing the identified biomarkers MASP-1 and VTN. The newly developed assay, successfully employed gold and silver nanoparticles as probes, which can differentiate between severe and non-severe dengue cases. This combination of TSP-1 with platelet counts and the point-of-care test using MASP-1 and VTN offers promising potential for rapid and convenient dengue severity detection in clinical settings.

Keywords: Dengue fever, LC-MS/MS, Lateral flow immunoassay (LFIA), Mannan-binding lectin-associated serine protease-1(MASP-1), Thrombospondin-1 (TSP-1), Vitronectin (VTN), Complement pathway.

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