# ABSTRACT

Dengue is a well-known viral infection caused by the Dengue Virus (DENV) which has antigenically distinct four serotypes DENV1, DENV2, DENV3, and DENV4. The co-circulation of multiple serotypes concurrently in a location increases the risk of severe dengue infection. The incidence geographical distribution of Dengue has increased dramatically over the past fifteen years and has become a major concern in tropical and sub-tropical countries. Factors like rapid urbanization, population growth, rise in temperature, and inadequate mosquito control practices have contributed significantly to this rise in Dengue cases. Dengue virus infections can result in a spectrum of illnesses from asymptomatic to Dengue fever (DF) and dengue hemorrhagic fever (DHF). Mild Dengue is characterized by sudden onset of fever accompanied by other symptoms like headache, and body ache whereas severe forms of dengue are presented with clinical conditions like vascular permeability, plasma leakage, thrombocytopenia, severe bleeding, or organ impairment. Severe dengue activates both the innate and adaptive immune systems, which in turn stimulate the production of cytokines. An over-production or skewed profile of cytokine expression which is generally known as cytokine storm is observed in DENV infection. This phenomenon has a direct effect on the vascular endothelial cells by increasing capillary permeability and causing leakage. Understanding the clinical symptoms and identifying key cytokines that may play an important role in the progression of the disease is crucial for prognosis. There is no specific treatment for dengue. Treatment focuses on relieving pain symptoms and initiation of rehydration therapy. Proper management of Dengue disease can be done by early and accurate case detection. The initial step in diagnosing a Dengue infection is based on clinical symptoms. A final and confirmatory diagnosis is made after proper laboratory testing is performed. Various laboratory diagnosis method includes the detection of i) virus by cell culture, ii) viral RNA iii) DENV antigens, and iv) specific antibodies to DENV. Serological testing developed to analyze antigen/antibody response during dengue infection is generally less expensive and easier to perform. Lateral Flow Assay had been the most commonly used point of care diagnostic tool for dengue virus infection. However, false positives due to antigenic cross-reactivity have

been reported between Dengue and Zika infections when using DENV NS1 antigen detection assays in acute cases. The development of a DENV-specific point-of-care diagnostic test that is immune to other flaviviruses is therefore critically necessary.

We designed our study to understand the cellular response and antibody response concerning natural Dengue virus infection. A hospital-based study was carried out with confirmed cases of dengue and non-dengue cases in the population of Assam. Samples were collected at collaborating hospitals Gauhati Medical College and Hospital and Tezpur Medical College and Hospital. Samples and clinical data were collected from collaborating hospitals after obtaining written informed consent and following SOPs /ICMR guidelines for sample collection.

We evaluated the profile of both inflammatory and anti-inflammatory cytokines (IL6, IL10, IL1β, IL12p70, and TNF) and chemokines (IL8, CXCL9, CXCL10, CCL2 and CCL5) in hospitalized NS1/IgM confirmed Dengue patients using Cytometric bead array (CBA) and bench top flow cytometry system. Increased levels of cytokines such as IL6, IL1β, and IL10 in Dengue patients when compared to healthy individuals indicated that Dengue infection activates the monocyte-neutrophil axis. Additionally, elevated levels of the chemokines CXCL9, CCL2, CXCL10, and IL8 promote the monocyte-neutrophil axis. Cell phenotyping study by Flow Cytometry indicates an increased frequency of intermediate monocytes and a significant decrease in the non-classical subset in dengue. While monocytes and neutrophils have CCL2 and IL8 receptors, T cells, NK cells, dendritic cells, and macrophages have CXCL10 and CXCL9 receptors (CXCR3) suggesting recruitment and involvement of these immune cells in DENV infection-mediated inflammation. In contrast to other chemokines, expression of CCL5 was higher in healthy control than in Dengue-positive patients. A positive correlation between Platelet count and CCL5 levels was observed in our study population. This observation is interesting considering that thrombocytopenia is an important clinical symptoms in Dengue infection.

Antibody response to DENV Envelope (E) and NS1 proteins was examined using peptide arrays of DENV1 E (NR50701), DENV2 E (NR510), DENV2 NS1(NR508), DENV3 E (NR511) and DENV3 (NR2753). Peptide arrays were obtained as a gift from the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH. We have employed the Indirect ELISA method to determine the seropositive peptides of the

whole Dengue peptide arrays. Peptides with high seropositivity in terms of frequency and titre and showing good antigenicity (>0.4) and conservancy (>80%) were considered to contain immunodominant epitopes and termed as immunodominant peptides. Simultaneously linear and conformational B cell epitopes of DENV Envelope and NS1 proteins were predicted using the Bioinformatics approach. B cell epitopes determined by the computational approach were seen to lie in immunodominant peptide region thus showing concordance in the two approaches used. A recombinant peptide construct (SP7) was designed and its diagnostic potential was evaluated using Dengue positive, Dengue negative, and *Japanese encephalitis* (J.E) positive sera. SP7 showed good diagnostics potential with greater than 90% sensitivity and specificity.

We have also designed a multi-epitope vaccine construct with potential B and T cell epitopes of DENV structural proteins i.e Envelope, prM and capsid. The vaccine construct was found to be antigenic with a 0.74 score and non-allergenic in nature. In silico immune simulation study demonstrated the potential of the vaccine contruct to be able to induce immunoglobulins and cytokines. Also, a steady increase in the concentrations of helper T cells, cytotoxic T cells, plasma B cells, and active B cells was predicted, indicating the vaccine's ability to produce a strong secondary immunological response and a robust immune memory.

**SCIENTIFIC PUBLICATIONS FROM THE THESIS**

1. Singha, S., Nath, N., Sarma, V. *et al.* Identification of Immunodominant Epitopes of Dengue Virus 2 Envelope and NS1 Proteins: Evaluating the Diagnostic Potential of a Synthetic Peptide. *Mol Diagn Ther* (2024). https://doi.org/10.1007/s40291-024-00728-8.

**SCIENTIFIC PRESENTATIONS FROM THE THESIS**

1. Singha, S and Baruah, S, Cytokine and Chemokine profile of Dengue virus infected participants from Assam, Northeast India, 44th Annual Conference of Indian Association of Medical Microbiologist, Microcon 2021, Guwahati, 22-24th
December 2021.
2. Singha, S and Baruah, S, Immunodominant peptides of Dengue virus Envelope and NS1 proteins overlap with predicted B-cell epitope regions, 48th Annual Conference of Indian Immunology Society (Immunocon-2022), Department of Molecular and Human Genetics Banaras Hindu University, Varanasi, 8th-9th July, 2022.
3. Singha, S and Baruah, S, Design of a novel multi-epitope-based subunit vaccine targeting structural proteins of Dengue virus using immuno-informatics approach, Golden Jubilee Conference of the Indian Immunology Society (Immunocon 2023), JL Auditorium, AIIMS, 5th -8th , October, 2023.

Signature of the Student

Sushmita Singha