CHAPTER I: INTRODUCTION

1.1 Introduction

Dengue is an important viral infection caused by Dengue virus (DENV) and is seen more frequently in tropical and subtropical countries. The World Health Organisation (WHO) has classified dengue as a major international public health problem since it is prevalent in the majority of tropical and sub-tropical nations[1]. Over the past few decades, there has been a notable increase in the prevalence of dengue case, rising from 505,430 cases in 2000 to 6.5 million cases in 2023, and is a burden in 195 countries [2].

Based on one modelling estimate, there are 390 million DENV infections per year, of which 96 million result in clinical symptoms. India's experience with dengue fever was less well-known than that of other Asian countries, including Vietnam, Thailand, and the Philippines. The first dengue fever case was documented in the Tamil Nadu district of Vellore in 1956, and the first case of severe dengue was reported in Calcutta (now Kolkata), West Bengal, in 1963[3]. Since then, reports of dengue epidemics have been documented from all around the nation[4-6]. Furthermore, persistent dengue outbreaks led to the creation of hyperendemic zones, which are essentially big, crowded cities where one or more of the four DENV serotypes are continuously circulating. To define the disease pattern in India, data on age and gender distribution, seasonality, and the annual report of cases and deaths from dengue are combined with, information on their regional distribution.

A number of variables including fast urbanization, population growth, rising temperatures and insufficient mosquito control measures are seen as confounding factors, for this concerning increase in the incidence rate[7-9]. Humans typically contract DENV through the bite of the primary vector *Aedes aegypti* mosquito, while reports of transmission by the secondary vector *A. albopictus* is also documented [10, 11]. Non-vector transmission of DENV can happen through blood transfusions, wounds, organ transplants and mucosal splashes [12-14]. Also, the first case of sexual transmission of dengue has been reported from Spain [15].

DENV is a single-stranded positive-sense RNA virus that has four different serotypes: DENV1, DENV2, DENV3, and DENV4 and belongs to the Flaviviridae family. DENV is encased in an icosahedral nucleocapsid, with a diameter of 50 nm[16]. With a genome size of 11 kb it is further encapsulated by membrane and viral envelope protein[17].Single polyprotein encoded by the DENV genome is splitted into seven non-structural proteins

(NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) and three structural proteins (the glycoproteins capsid (C), membrane (M), and envelope (E))[18, 19]. All four dengue serotypes share a similarity of 65% of their genome and also cause nearly identical symptoms in humans and co-circulation of more than one serotype simultaneously in a region is a risk of severe dengue infection[20, 21].

The DENV replication cycle starts when the virus enters the cell through a variety of host cell receptors or when the virus-antibody immune complex's Fc region binds to Fc receptors on the target host cell. DENV penetrates the cell via binding to receptors on the host cell. Through receptor-mediated endocytosis, internalisation takes place, creating an early endosome. As the pH inside the early endosome drops, conformational changes occur, causing the nucleocapsid to be released into the cytoplasm, resulting in genome uncoating [22]. Following that, the viral RNA replication starts using the resources of the host cell. The virus utilised the ribosomes located in the host's rough endoplasmic reticulum (ER) to translate its viral RNA and produce its viral polypeptide which is then cleaved into three structural and seven non-structural proteins. The C proteins encapsulate the freshly synthesized viral RNA, creating a nucleocapsid. The M and E proteins surround the nucleocapsid in the rough endoplasmic reticulum (ER)after which envelope and outer layer are added. This non-infectious and immature form of virus converts into its infectious form after passing through the Golgi apparatus. In Golgi, immature viral particles undergo structural changes due to acidification before being exposed to furin protease to generate mature viral particles. The replication cycle of mature virus particles is completed when they are exocytosed into the extracellular matrix. The mature viruses are then released from the cell and have the ability to infect more cells [23]

A wide variety of clinical symptoms, from asymptomatic to dengue fever (DF) or severe dengue (SD) can be caused by DENV, [24]. Patients with dengue fever experience symptoms such as vomiting, headaches, fever, stomach pain, and maculopapular rash after an incubation period of 4–7 days[25, 26]. Severe dengue cases have been reported to exhibit symptoms like thrombocytopenia, vascular permeability, plasma leakage, severe bleeding, or organ dysfunction; however, severe dengue is a condition that only worsens after the virus has left the body and is probably related to immunopathology[27,

28]. Dengue has no particular treatment; however, infections can be controlled with early case identification, appropriate care, and the start of rehydration therapy[29].

The increasing prevalence of dengue fever necessitates the development of effective treatments as no specific treatment for Dengue is known. Efforts to create a dengue vaccine or a treatment that works effectively to treat the disease have not been successful thus far. The requirement to offer protection against all four dengue serotypes to prevent antibody-dependent enhancement in future infections has made the development of dengue vaccines challenging. There are numerous approaches being used to generate dengue vaccines, such as recombinant proteins, chimeric live attenuated viruses, inactivated viruses, and live attenuated viruses[30]. Currently, several vaccine candidates are going through different phases of clinical studies. Tetravalent live attenuated chimeric dengue virus vaccine "DENGVAXIA," produced by Sanofi Pasteur, is the first licensed dengue vaccine and is based on the yellow fever (YF) 17D vaccine strain. The four dengue serotypes' genes were substituted for the YF vaccine's pre-membrane (prM) and envelope (E) proteins to form the four monovalent chimeric vaccine viruses, or CYD1-4. These four monovalent viruses are combined into one vaccine preparation to create the tetravalent CYD1-4 dengue vaccine (TDV)[31].

In response to DENV infection, the adaptive immune system helps prevent re-infection but also contributes to the enhancement of the disease. The primary immune response to DENV is quite typical, involving an early IgM response to DENV antigens followed by an IgG response whereas secondary infection, is characterized by an accelerated IgG response and a decreased IgM response[32, 33]. Precursor membrane (pre-M), non-structural protein 1 (NS1), and envelope (E) are the main targets of the antibody response to dengue virus infection in humans. However, non-structural proteins like NS3 and NS5 have also been linked to weak antibody responses [34]. The primary surface component of the dengue virion, the Envelope glycoprotein, has been most extensively analyzed for B cell epitopes. DENV E protein comprises three domains, namely domain I, domain II, and domain III. There are many epitopes within each of the three domains that are bound by antibodies. Serological response to the E protein in humans is highly serotype cross-reactive. The majority of human monoclonal antibodies specific to the E protein were found to bind to multiple dengue virus serotypes. The E protein's domain III has the

highest variation in amino acid sequence amongst serotypes; it contains the potential receptor-binding domain that enables the virus to attach to host cells. Thus, antibodies targeted for this domain is highly serotype-specific. According to studies, people who have already had dengue are more likely to have severe dengue. When a secondary infection occurs, the dengue virus enters the host cell through the Fc receptor with the help of a cross-reactive neutralizing antibody. This phenomenon is termed "antibody-dependent enhancement" (ADE).ADE is essential for the invasion of host immune cells and the progression of illness (18). The majority of dengue-specific antibodies in human sera bind to numerous DENV serotypes and are weakly neutralizing; only a small percentage of all DENV-specific antibodies potently and type-specifically neutralize DENV, making the immune response to DENV protection debatable. Considering this a potent vaccine should have the ability to induce neutralizing antibodies against each of the dengue serotypes.

Dengue virus is tropic and known to infect human dendritic cells ,monocytes, and macrophages, which are known to produce cytokines. A "cytokine storm"—a cascade of cytokine production—is created when the immune system is overstimulated, which increases vascular permeability. Studies has also demonstrated that proinflammatory, antiviral, and proinflammatory cytokines are produced as a result of the interaction between DENV and host cells. IL-8 was produced by DENV-infected macrophages and endothelial cell. DENV-infected endothelial cells also secretes CXCL10, CXCL9, IL-6, and CCL5 [35], These mediators have chemoattractant qualities and can increase permeability, which may lead to inflammation and plasma leakage. Research has shown that individuals with dengue hemorrhagic fever had higher levels of pro-inflammatory and vasoactive cytokines before and during plasma leakage, and the degree of elevation is correlated with the severity of the disease. A better understanding of the immune responses linked to elevated or lowered risk for severe dengue will be crucial for the clinical trials of potential multivalent DENV vaccines that are expected to occur in the coming years.

Proper management of Dengue depends on laboratory diagnosis, which also enables early patient treatment and epidemic prevention. Clinical symptoms are the basis for the preliminary diagnosis. There are several ways to diagnose DENV infection, such as using cell culture, NAAT to identify the virus's nucleic acid, DENV antigens, and particular

DENV antibodies. Even though virus isolation offers the most precise test, virus culture necessitates a biosafety level 2 laboratory, a lot of time and effort, and expert staff training. Serum samples are stable under these climate circumstances, and serological testing, which was created to analyze antigen/antibody reactions during dengue infection, is often less expensive and easier to execute. Until recently, the most widely used point-of-care diagnostic technique for dengue virus infection was a commercial Rapid Diagnostic Test (RDT) kit. However, when DENV NS1 antigen detection assays are used in acute cases, false positives caused by antigenic cross-reactivity between Dengue and Zika infections have been recorded[36]. Cross-reactivity was also reported between DENV and SARS-COV-2 antibodies based on rapid serological tests[37]. The development of a DENV-specific point-of-care diagnostic test that is immune to other flaviviruses is therefore critically necessary.

To address the problems associated with Dengue infection we have focused our study on two aspects: cellular response and antibody response in natural DENV infection. Broadly the following objectives were defined-

1. Characterisation of cytokines and chemokines levels in serum of Dengue infected patients.
2. Determination of immunodominant peptides using whole peptide array of Dengue Envelope and NS1 proteins of the Dengue serotypes.
3. a) Bioinformatic analysis of Dengue Envelope and NS1 proteins to identify potential T-cell and B-cell epitopes

3. b) Epitope-based subunit vaccine construct for Dengue virus using immuno-informatics approach

4. Evaluation of identified epitopes for Dengue virus antibody diagnostic assay.

Investigating the cellular and cytokine response to Dengue virus infection to determine key cytokines and chemokines specific to DENV infection was one of the goals of the study. Using Cytometric Bead Array technology we have checked the serum cytokine/chemokine protein expression profiles of dengue-infected participants and analyzed the correlation of these proteins with the clinical data collected. Cytokines such as IL6, IL1β, and IL10 was found to be elevated in Dengue patients in comparison to healthy individuals. Elevated levels of IL8, CXCL9, CCL2, and CXCL10 chemokines were also observed in Dengue-positive sera. In contrast to other chemokines, expression

of CCL5 was higher in healthy control than in Dengue-positive patients Interestingly both cytokines and chemokines reported to show increased expression supported monocyte-neutrophil axis. This could be used as a prognostic marker of dengue disease. Platelet count in Dengue is a very important factor hence a correlation analysis was performed with platelet count of Dengue patients and their cytokine/chemokine expression levels where a positive correlation of platelet count with chemokine CCL5 was observed. Cell phenotyping study by Flow Cytometry indicates an increased frequency of intermediate monocytes and a decreased in the non-classical monocyte subset in dengue.

We have identified epitopes on DENV Envelope and NS1 proteins using both serology and bioinformatics approaches An Indirect ELISA method was used to analyze the antibody reaction to the DENV Envelope and NS1 proteins using peptide arrays. Immunodominant peptides were determined as peptides that exhibit high levels of seropositivity in terms of frequency and titer, as well as good antigenicity (>0.4) and conservancy (>80%). These peptides are thought to contain immunodominant epitopes. For computational identification of epitopes, we have used Immune Epitope Database and Analysis Resource (IEDB)-based servers to predict the linear and conformational B cell epitopes of DENV Envelope and NS1 proteins. We have overlapping peptide regions identified as epitopes in both approaches. Concurrence results obtained in both approaches indicating agreement between the two methods are the strength of the study. Two best epitopes were selected to design a recombinant synthetic peptide (SP7). The diagnostic capability of the recombinant peptide construct (SP7) was assessed using sera from patients with Dengue, Dengue negative, and J.E. positive infections. SP7 had strong diagnostic potential, exhibiting a sensitivity and specificity of over 90%.

Targeting epitopes in viral antigens is important for several practical applications, such as the development of diagnostics and the creation of vaccinations based on epitopes. Moreover, we have also designed multi-epitope vaccine construct with potential B and T cell epitopes of DENV structural proteins i.e Envelope, prM and capsid. Vaccine construct was found to be antigenic with 0.74 score and non-allergenic in nature. In silico immune simulation study demonstrated the potential to immunoglobulins and cytokines. Also a steady increase in the concentrations of cytotoxic T cells, helper 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

1.2 References

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