CHAPTER IV: DETERMINATION OF IMMUNODOMINANT PEPTIDES AND POTENTIAL B AND T CELL EPITOPES OF ENVELOPE AND NS1 PROTEINS OF THE DENGUE SEROTYPES.

4.1 Introduction

Dengue Virus (DENV) infection is a globally important vector (*Aedes aegypti and Aedes albopictus* mosquitoes*)* borne disease, with approximately 50% of the world's population at risk of dengue (100-400 million infections/ year) where 70% of the global burden is from Asia[1].

DENV has been in circulation in the Indian subcontinent since the 1950s. All four serotypes have been reported in India [2]. Being a tropical country India has experienced a high level of transmission during the last five years and more than one hundred thousand dengue cases have been reported annually. It is anticipated that the co-circulation of multiple DENV serotypes may continue and could become a potential threat to cause an outbreak again. For Northeast India, there has been a gradual increase in Dengue cases in the last few years which is consistent with the rapid urbanization of this region[3] and all four serotypes have been reported [4].

Clinically DENV infection is difficult to distinguish from other virus infections(*Japanese encephalitis* (JEV), Zika, Chikungunya etc) and needs laboratory testing[5]. Available confirmatory tests include viral antigen or nucleic acid detection, viral gene detection by reverse transcription-polymerase chain reaction (RT-PCR) and serology by Enzyme-linked immunosorbent assay (ELISA), and Lateral Flow assay (LFA) and mainly target DENV1 and DENV2. DENV antigen or nucleic acid is detected in the early phase of the disease, and subsequently, serology is the preferred method for ease of performance and stability in the tropical climate[6]. However cross-reactive antibodies become a concern in serology-based diagnosis. Identification and targeting of specific epitopes in a synthetic construct could overcome the issue of cross-reactivity and enhance the sensitivity of the assay.

DENV is an RNA virus belonging to family *Flaviviridae* with four distinct serotypes DENV1, DENV2, DENV3 and DENV4 (7). Genome size is 11kb which is packed inside an icosahedral nucleocapsid. DENV genome encodes for a single polyprotein and is further cleaved into three structural and seven non-structural proteins [7].

Dengue Envelope (E) protein is a glycoprotein (two N-linked glycosylation sites, at Asn-67 and Asn-153) approximately 55 kDa which is involved in host cell receptor recognition and attachment. Many studies have shown that most neutralizing antibodies recognize the E protein of DENV. E protein consists of three structural domains D-I, D-

II and D-III, The Receptor binding domain falls within Domain III so, antibodies against E- protein have a great potential for early protection[8]. Domain III is immunoglobulin-like in structure and is exposed and accessible on the virion surface. Studies using monoclonal antibodies (mAbs) have shown the presence of neutralizing epitopes in all three domains of the envelope protein, however primary interaction of neutralizing Abs and surface exposed loop of D-III was seen[9].

Non-structural (NS) proteins of DENV have their specific part in the pathogenesis of the disease. Among all the non-structural proteins, DENV NS1 is mostly studied because of its dual function in immune evasion and genome replication. DENV NS1 is a highly conserved 46kDa glycoprotein and contains 12 conserved cysteine (Cys) residues and two glycosylation sites (Asn-130 and Asn-207). Cell infected with the virus secretes a hexameric form of NS1 which can be found in the blood from the first day of symptoms[10]. Levels of NS1 correlate with the onset of haemorrhagic fever. Studies also have reported the role of NS1 in the disruption of endothelial glycocalyx which increases permeability and results in vascular leakage[11].

Mapping of epitopes on DENV E and NS1 proteins is necessary to determine potential peptides for diagnostics purposes. We aimed to identify immunodominant regions of DENV E and NS1 proteins in naturally infected patients and comparing with B cell epitopes identified by the in-silico approach. Using a peptide array of DENV E and NS1 proteins we have initially determined the immunodominant regions of these proteins detected by patient sera and simultaneously we have cross-checked the B cell epitope regions of these viral proteins. Results of our study show a concurrence between the two approaches followed and were successful in determining DENV-specific IgG antibodies.

4.2 Materials and method

Identification of potential epitopes and immunodominant regions of DENV E and NS1 proteins was carried out following two different approaches. The first approach was a prediction of B cell linear and conformational epitopes using bioinformatics tools. Secondly, a serology-based study was carried out using peptide arrays of viral proteins.

4.2.1 Peptide array and retrieval of the protein sequences

For this study, we have screened the whole peptide array of DENV1 E(NR50701), DENV2 E (NR510) [12] and NS1(NR508) [13, 14]13, 14], DENV3 E (NR511) and DENV3 NS1(2753) proteins. The peptide arrays reagents were obtained as a gift from the

NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH). The Peptide array of DENV1, DENV2 and DENV3 E consisted of 84,67 and 68 peptides respectively and the peptide array of DENV2, DENV3 NS1 array consisted of 47 and 60 peptides respectively. Peptides are 15- to 20-mers, with 10 or 12 amino acid overlaps. Corresponding full-length amino acid sequences of DENV1 Envelope (GenPept: P33478), DENV2 Envelope (GenPept: AAA42962) and NS1 (GenPept: AAA42941), DENV3 Envelope (GenPept: AAT69740). And NS1 (GenPept: AAA99437). were retrieved from the protein database of NCBI.

4.2.2 Identification of B cell epitopes using bioinformatics

4.2.2.1 Prediction of linear B cell epitopes

B cell linear epitopes were predicted by BepiPred2.0[15] for Dengue virus E and NS1 protein. Prediction scores of more than 0.5 were considered as B cell epitopes and epitopes shorter than 5 amino acid length were excluded. Parameters like antigenicity, surface accessibility, turns, flexibility, and hydrophilicity were considered. The BepiPred-2.0 server uses a Random Forest algorithm trained on epitopes and non-epitope amino acids to predict B-cell epitopes from a protein sequence.

4.2.2.2 Prediction of conformational B cell epitopes

A conformational or discontinuous B-cell epitope is located closely to the protein's three-dimensional (3D) structure but is discontinuous in the protein sequence. Discontinuous epitopes were predicted using ElliPro [16] for DENV2 E and NS1 proteins following default parameters. ElliPro uses 3D protein structures to predict B cell epitopes.

4.2.3 Determining seropositivity and titre by Indirect ELISA

We have analysed the antibody response to Dengue virus E and NS1 whole peptide array by Indirect ELISA method in 50 DENV-positive and 5 healthy individuals. Checker-board ELISA procedure was employed to standardise the assay using different concentrations (1:100,1:250.1:500) of DENV Env monoclonal antibody DENV1(NR-4746), DENV2(NR-2556) and DENV3(NR-15526) and peptide (0.5 to 1.5ng/ µl). Thereafter, the optimum concentration of antibody(1:250) and peptide(1ng/ µl) was

selected. For Indirect ELISA we have used Nunc Maxisorp 96-well polystyrene plates. After resuspension of the peptides in their respective solvent, 50µl of each peptides were coated independently at 1ng/µl concentration in coating buffer (Carbonate-bicarbonate pH 9.6) in duplicates and the plates were kept overnight at 4°C. Unbound peptides were washed off using wash buffer (PBS pH 7.4 with 0.05% Tween 20 -PBST) and 100µl of Blocking buffer (5% BSA in PBS pH 7.4) were added to each well followed by incubation at room temperature for 3 hours. The plates were washed three times with wash buffer followed by the addition of 50µl diluted serum in each well (serum dilution ratio 1:250 in PBS) and incubated for 1 hour at 37°C. After washing the plates three times HRP tagged Anti-human IgG antibody (ab97225) was added in each well and incubated for 1 hour at 37°C. the unbound antibodies were washed off three times using a wash buffer. 50µl of TMB substrate(Sigma-T0440) was added in each well followed by 20 minutes incubation at room temperature protected from light. The peroxidase reaction was stopped by adding 50µl of 1 N H2SO4 in each well. Absorbance was checked at 450nm. Cut-off values for positive detection were determined using the formula Cut-off = Mean OD of negative control samples + 3 standard deviations (S.D).

4.2.4 Criteria for selection of immunodominant peptides

From the whole peptide array, peptides with antibody titre above cut-off were initially selected. Thereafter, the frequency of seropositive patients with antibody titres greater than or equal to 1.5 Arbitrary Units (A.U) was checked in the studied samples. The frequency of seropositivity and titre were considered as criteria for the selection of immunodominant peptides. Arbitrary Unit (A.U) was calculated using the formula.

A.U=$(Sample\ mean - experimental\ mean)/(Control\ mean)$

Where, Sample mean = Peptide absorbance value of patient sample - blank value

Control mean = Peptide absorbance value of non-Dengue control sample – blank value, Experimental mean = Negative control mean + 3 x Standard Deviations – blank readings). Here in Negative control, no primary antibody /serum was added.

4.2.5 Conservancy analysis and antigenicity

We have used the IEDB platform to predict the conservancy of immunodominant peptides predicted in our study. A sequence identity of 100% was used as a threshold to check the conservancy of epitopes across 50 different DENV intrastrains from across the world. The Conservancy was also checked with 200 pan DENV serotype strains 50 JEV, and 50 Zika virus strains using an 80% sequence identity match. The antigenicity of the immunodominant peptides was checked by VaxiJen 2.0 server[17].

4.2.6 Surface Localization of Immunodominant Peptide Sequence in Proteins.

The crystal structures of DENV1E(PDB Id-3G7T) , DENV2 E (PDB Id-3J2P) and NS1 (PDB Id-7WUR) DENV3 E(PDB Id-7A3P) were downloaded from RCSB PDB database [18]. Localization of the immunodominant peptides on DENV whole E and NS1 proteins was done using PyMOL 2.5.4 software[19]. Visualization of all the selected peptides in whole proteins was achieved by colour-coding the specific amino acid residues.

4.2.7 Prediction of T cell epitopes

IEDB based methods NetMHC pan 4.1 EL and NetMHCII 4.1 E.L[20] were used to predict 9-mer peptide length cytotoxic T lymphocytes (CTL) epitope and T helper cell (HTL) epitopes of 15-mer length respectively. 27 HLA supertype alleles with maximum population coverage (approximately>97-99%) were selected to identify and predict MHC I and MHC II binding epitopes.

4.3. Results

4.3.1 Immunodominant epitopes of DENV proteins

4.3.1.1 Immunodominant peptide of DENV1 Envelope determination using peptide array.

Using indirect ELISA, the natural antibody response to DENV1 E peptide array was determined in serum samples from DENV-positive patients. Initial screening based on antibody titre greater than or equal to 1.5 yielded 19 seropositive peptides from a panel of 84 peptides. 47% (9 peptides) i.e E1P2, E1P7, E1P13, E1P14, E1P15, E1P17, E1P23, E1P24, E1P33 of the seropositive peptides were found to be seropositive with a frequency greater than 0.9 as shown in Figure 4.1. These dominantly recognised peptides were considered to contain immunodominant regions and termed immunodominant peptides

of DENV1 Envelope protein.

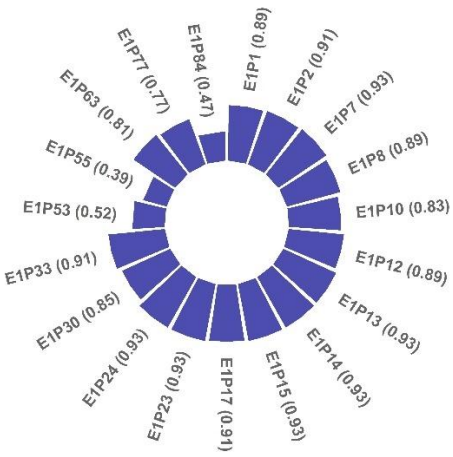
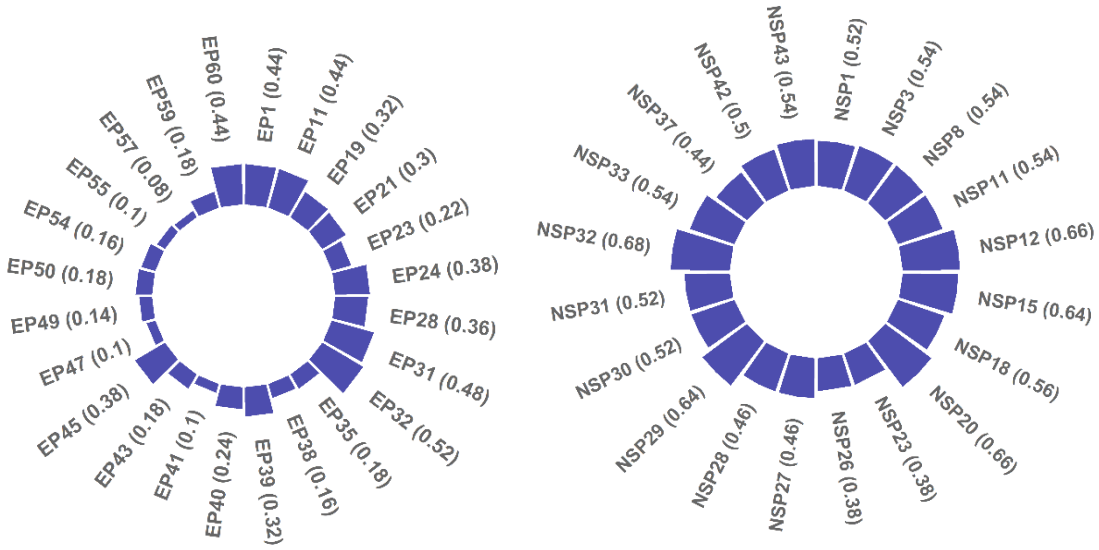


Figure 4.1: Seropositive peptides of DENV 1 Envelope peptide array. Here the frequency of patients recognizing the peptides with greater than or equal to 1.5 A. U is shown in a bracket.

4.3.1.2 Immunodominant peptide of DENV 2 Envelope and NS1 protein determination using peptide array.

Natural antibody response to DENV2 E peptides in serum samples of DENV-positive individuals was measured by Indirect ELISA. First screening criteria based on antibody titre greater than or equal to 1.5 yielded 24 seropositive peptides from a panel of 67 peptides array. Further frequency of seropositivity of these 24 seropositive peptides in dengue-positive serum samples was analysed and 33% (8 peptides) of the shortlisted peptides showed a frequency greater than 0.35 (Figure 4.2 A). These dominantly recognised peptides were considered to contain immunodominant regions and termed immunodominant peptides of DENV2 Envelope protein. Peptides EP1, EP11, EP31, EP32 and EP60 showed frequency greater than 0.4 whereas, EP24, EP28 and EP45 showed frequency greater than 0.35.



A B

Figure 4.2: Seropositive peptides of DENV2 A) Envelope peptide array NR510 and B) NS1 peptide array NR508. Here the frequency of patients recognizing the peptides with greater than or equal to 1.5 A. U is shown in a bracket.

Similarly, the peptide array for Dengue 2 Non-structural 1 protein was screened. Based on antibody titre (greater than or equal to 1.5) 20 seropositive peptides were selected from a panel of 47 peptide arrays. 30% (6 peptides) of the seropositive peptides were found to be seropositive with a frequency greater than 0.56 as shown in Figure 4.2 B. The frequency of seropositive patients for peptides NSP12, and NSP20, was found to be 0.66, for peptides NSP15 and NSP29 was 0.64 whereas the frequency for peptides NSP18 was 0.56 and for NSP32 was 0.68. These 6 dominantly recognised peptides were considered to contain immunodominant regions and termed immunodominant peptides of DENV2 NS1 protein.

4.3.1.3 Immunodominant peptide of DENV 3 Envelope and NS1 protein determination using peptide array.

Using the same criteria of seropositivity and frequency, 7 immunodominant peptides of DENV3 E and 5 immunodominant peptides of DENV3 NS1 were determined. Among DENV3 E peptides E3P3 and E3P44 showed a frequency greater than 0.46 whereas the frequency of DENV3 NS1 peptides NS3P5, NS3P29, NS3P30, NS3P34 and NS3P38 were found to be greater than 0.6 (Figure 4.3).

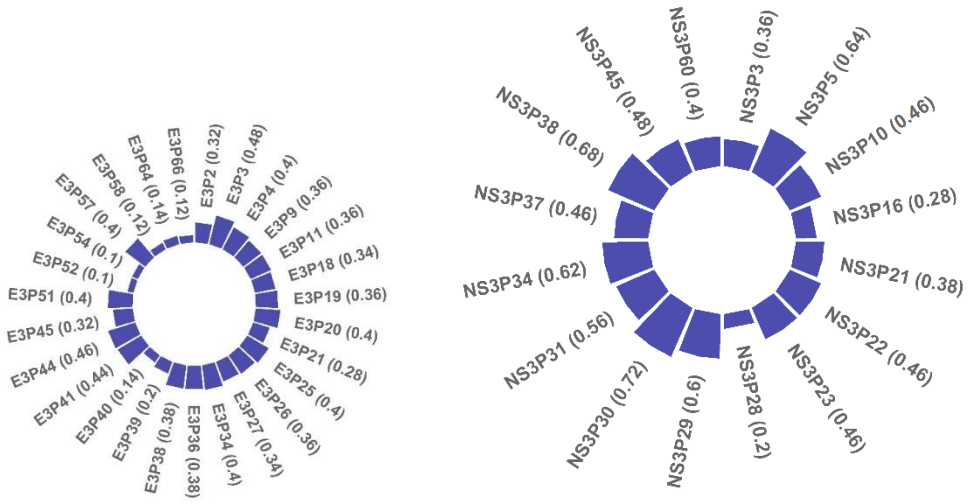


Figure 4.3: Seropositive peptides of DENV3 A) Envelope peptide array NR511 and B) NS1 peptide array NR2753. Here the frequency of patients recognizing the peptides with greater than or equal to 1.5 A. U is shown in a bracket.

4.3.2 B cell epitope mapping by immunoinformatics approach

4.3.2.1 Prediction of B cell linear epitope of DENV1 Envelope and NS1 protein

8 B cell linear epitopes were predicted for Dengue virus 1 E protein and 7 linear epitopes for NS1 proteins (Table 4.1). The antigenicity of each linear B cell epitopes were checked and 10 epitopes were found to have acceptable antigenicity considering the VaxiJen 2.0 server threshold score of 0.4.

Protein	Sl No	Start	End	Sequence	Length	Vaxijen score	Antigenicity
DENV1 Envelope	**1**	**6**	**13**	IGNRDFVE	**8**	**0.7460**	**Antigenic**
	2	**48**	**55**	TEVTNPAV	**8**	**0.4133**	**Antigenic**
	3	**68**	**103**	TTTDSRCPTQGEATLVEEQDTNFVCRRTLVDRGWGN	**36**	**0.7371**	**Antigenic**
	4	**146**	**159**	GDQHQVGNETTEHG	**14**	0.1592	**Non Antigenic**

	5	**211**	**238**	QWFLDLPLPWTSGASTSQETWNH QDLLV	**28**	**0.4254**	**Antigenic**
	6	**306**	**318**	FKLEKEVAETQHG	**13**	0.3495	**Non-antigenic**
	7	**386**	**397**	ALKLSWFKKGSS	**12**	**0.8333**	**Antigenic**
	8	**419**	**427**	AWDFGSIGG	**9**	**1.7867**	**Antigenic**
DENV1 NS1	**1**	**26**	**39**	HTWTEQYKFQADSP	**14**	-0.3843	**Non-Antigenic**
	2	**93**	**130**	VSGILAQGKKMIGPQPMEHKYS WKSWGKAKIIGADVQN	**38**	**0.5882**	**Antigenic**
	3	**139**	**150**	NTPECPDDQRAW	**12**	0.0981	**Non-Antigenic**
	4	**228**	**243**	SHTLWSNGVLESEMII	**16**	0.0488	**Non-Antigenic**
	5	**248**	**272**	248GGPISQHNYRPGYFTQTAGPW HLGK272	**25**	1.0218	**Antigenic**
	6	**289**	**318**	289EHCGNRGPSLRTTTVTGKIIHE WCCRSCTL318	**30**	0.4127	**Antigenic**
	7	**338**	**345**	338VKDKEENL345	**8**	1.4969	**Antigenic**

Table 4.1- B cell linear epitopes of DENV1 Envelope and NS1 protein predicted by BepiPred 2.0. Antigenicity score by VaxiJen 2.0 (threshold>0.4).

4.3.2.2 Prediction of B cell linear epitope of DENV2 Envelope and NS1 protein

A total of 11 B cell linear epitopes were predicted for Dengue virus 2 E protein and 8 linear epitopes for NS1 proteins (Table 4.2). The antigenicity of each linear B cell epitope was checked and 10 epitopes were found to have acceptable antigenicity considering the VaxiJen 2.0 server threshold score of 0.4.

Protein	Sl No	Start	End	Sequence	Length	Vaxijen score	Antigenicity
DENV2 Envelope	1	5	9	GISNR	5	-	-
	2	48	55	TEAKQPAT	8	**0.2204**	Non antigenic
	3	68	103	TTTDSRCPTQGEPTLNEEQDKRFVCKHSMVDRGWGN	36	**0.5708**	**Antigenic**
	4	145	159	SGEEHAVGNDTGKHG	15	**0.0018**	Non Antigenic
	5	169	173	SITEA	5	-	-
	6	213	235	FLDLPLPWLPGADTQGSNWIQKE	23	**0.4451**	**Antigenic**
	7	269	273	EIQMS	5	-	-
	8	303	317	TGKFKVVKEIAETQH	15	**-0.0626**	Non Antigenic
	9	339	345	IMDLEKR	7	**1.7198**	**Antigenic**
	10	386	397	QLKLDWFKKGSS	12	**0.5635**	**Antigenic**
	11	420	427	WDFGSLGG	8	**2.1175**	**Antigenic**
DENV2 NS1	1	26	39	HTWTEQYKFQPESP	14	**0.1899**	Non-Antigenic
	2	93	130	IKGIMQAGKRSLQPQPTELKYSWKTWGKAKMLSTESHN	38	**0.7985**	**Antigenic**
	3	140	148	TAECPNTNR	9	**0.7593**	**Antigenic**
	4	173	179	EKQDVFC	7	**2.6007**	**Antigenic**
	5	228	243	SHTLWSNGVLESEMII	16	**0.0488**	Non Antigenic
	6	248	272	AGPVSQHNYRPGYHTQTAGPWHLGK	25	**0.9172**	**Antigenic**
	7	289	318	EDCGNRGPSLRTTTASGKLITEWCCRSCTL	30	**0.2844**	Non-Antigenic
	8	338	344	LKEKEEN	7	**1.5819**	**Antigenic**

Table 4.2- B cell linear epitopes of DENV2 Envelope and NS1 protein predicted by BepiPred 2.0. Antigenicity score by VaxiJen 2.0 (threshold>0.4).

4.3.2.3 Prediction of B cell linear epitope of DENV3 Envelope and NS1 protein

7 B cell linear epitopes were predicted for Dengue virus 3 E protein and 6 linear epitopes for NS1 proteins (Table 4.3). The antigenicity of each linear B cell epitopes were checked and 10 epitopes were found to have acceptable antigenicity considering the VaxiJen 2.0 server threshold score of 0.4.

Protein	Sl No	Start	End	Sequence	Length	Vaxijen score	Antigenicity
DENV3 Envelope	1	6	13	6VGNRDFVE13	8	**0.4661**	**Antigenic**
	2	67	103	67NITTDSRCPTQGEAVLPEEQDQNYVCKHTYVDRGWGN103	37	**0.6026**	**Antigenic**
	3	146	157	146GDQHQVGNETQG157	12	0.1322	Non-Antigenic
	4	211	237	211FFDLPLPWTSGATTETPTWNRKELLV237	26	**0.5982**	**Antigenic**
	5	267	275	267EIQNSGGTSI275	10	**0.4023**	**Antigenic -**
	6	304	315	304FVLKKEVSETQH315	12	**0.428**	**Antigenic**
	7	384	395	384ALKINWYKKGSS395	12	**0.9740**	**Antigenic**
DENV3 NS1	1	24	39	24HTWTEQYKFQADSP39	14	-0.3843	Non-Antigenic
	2	91	117	91ITGVLEQGKRTLTPQPMELKYSWKTWG117	27	0.9619	**Antigenic**
	3	226	241	226SHTLWSNGVLESDMII241	16	-0.0617	Non-Antigenic
	4	246	280	246AGPISQHNHRPGYHTQTAGPWHLGKLELDFNYCEG280	35	**0.8141**	**Antigenic**
	5	288	316	288NCGTRGPSLRTTTVSGKLIHEWCCRSCTL316	29	**0.4677**	**Antigenic**
	6	335	345	335PINEKEENMVK345	11	**0.666**	**Antigenic**

Table 4.3- B cell linear epitopes of DENV3 Envelope and NS1 protein predicted by BepiPred 2.0. Antigenicity score by VaxiJen 2.0 (threshold>0.4).

4.3.2.4 Prediction of B cell linear epitope of DENV4 Envelope and NS1 protein

7 B cell linear epitopes were predicted for Dengue virus 4 E protein and 8 linear epitopes for NS1 proteins (Table 4.4). The antigenicity of each linear B cell epitopes were checked and 8 epitopes were found to have acceptable antigenicity considering the VaxiJen 2.0 server threshold score of 0.4.

Protein	Sl No	Start	End	Sequence	Length	Vaxijen score	Antigenicity
DENV4 Envelope	1	67	102	67ITTATRCPTQGEPYLKEEQDQQYICRRDVVDRGWGN102	36	0.3870	Non antigenic
	2	145	157	145GDTHAVGNDISNH157	13	-0.3526	Non antigenic
	3	211	238	211WFLDLPLPWAAGADTSEVHWNYKERMVT238	28	**0.8989**	**Antigenic**
	4	268	275	268EVDSGDGN275	8	**0.8081**	**Antigenic**

	5	304	317	304KFSIDKEMAETQHG317	14	-0.1173	Non antigenic
	6	388	396	388LHWFRKGSS396	9	0.7048	**Antigenic**
	7	419	426	419WDFGSVGG426	8	**2.24**	**Antigenic**
DENV4 NS1	1	25	38	25HTWTEQYKFQPESP38	14	0.1899	Non-Antigenic
	2	92	113	92VKGVLSKGKRALAPPVNDLKYS113	22	0.6759	**Antigenic**
	3	122	129	122IFTPEAKN129	8	**0.785**	**Antigenic**
	4	137	147	137PDTSECPNER147	10	-0.0319	Non antigenic
	5	171	178	171REGSSEVC178	8	-0.2152	Non Antigenic
	6	227	240	227THTLWSNGVLESQM240	14	0.1948	Non antigenic
	7	248	271	248GPFSQHNYRQGYATQTVGPWHLGK271	24	**0.8183**	**Antigenic**
	8	289	317	289DCDHRGPSLRTTTASGKLVTQWCCRSCTM317	29	**0.4576**	**Antigenic**

Table 4.4- B cell linear epitopes of DENV4 Envelope and NS1 protein predicted by BepiPred 2.0. Antigenicity score by VaxiJen 2.0 (threshold>0.4).

4.3.3 B cell conformational epitope prediction

Discontinuous or conformational B cell epitopes were predicted using the IEDB-based web server ElliPro for DENV E and NS1 proteins. The default threshold parameters such as a maximum distance of 6 Angstrom and a minimum score of 0.5 were taken into consideration.

4.3.3.1 B cell conformational epitope prediction of DENV1

B cell conformational epitopes of DENV1 E and NS1 proteins are given in the following table 4.5

Protein	No.	Residues	No of residues	Score
DENV1 Envelope	1	A:V428, A:S431, A:V432, A:G433, A:K434, A:L435, A:V436, A:H437, A:Q438, A:I439, A:F440, A:G441, A:T442, A:A443, A:Y444, A:G445, A:V446, A:L447, A:F448, A:S449, A:G450, A:V451, A:S452, A:W453, A:T454, A:M455, A:K456, A:I457, A:G458, A:I459, A:G460, A:I461, A:L462, A:L463, A:T464, A:W465, A:L466, A:G467, A:L468, A:N469, A:S470, A:R471, A:S472, A:T473, A:S474, A:L475, A:S476, A:M477, A:T478, A:C479, A:I480, A:A481, A:V482, A:G483, A:M484, A:V485, A:T486, A:L487, A:Y488, A:L489, A:G490, A:V491, A:M492, A:V493, A:Q494, A:A495	66	0.82
	2	A:E62, A:A63, A:K64, A:I65, A:S66, A:N67, A:T68, A:T69, A:T70, A:D71, A:S72, A:R73, A:C74, A:P75, A:T76, A:Q77, A:G78, A:E79, A:A80, A:T81, A:L82, A:V83, A:E84, A:E85, A:Q86, A:D87, A:A88, A:N89, A:F90, A:V91, A:C92, A:R93, A:R94, A:T95, A:F96, A:V97, A:D98, A:R99, A:G100, A:W101, A:G102, A:N103, A:G104, A:C105, A:G106, A:L107, A:F108, A:G109, A:K110, A:G111, A:S112, A:L113, A:I114, A:T115, A:C116, A:A117, A:K118, A:F119, A:K120, A:V122, A:T123, A:E229, A:T230, A:W231, A:R233, A:D235, A:L237, A:V238, A:T239, A:F240, A:K241, A:T242, A:A243, A:H244, A:A245, A:K246, A:K247, A:Q248, A:E249, A:V250, A:V251, A:V252, A:L253, A:G254, A:S255, A:Q256	86	0.756
	3	A:S16, A:G17, A:A18, A:T19, A:P39, A:E172, A:Q174, A:L175, A:T176, A:D177, A:Y178, A:G179, A:A180, A:M289, A:D290, A:K291, A:L292, A:T293, A:L294, A:K295, A:G296, A:M297, A:S298, A:Y299, A:V300, A:M301, A:C302, A:T303, A:G304, A:S305, A:F306, A:K307, A:L308, A:E309, A:K310, A:E311, A:V312, A:V322, A:Q323, A:V324, A:K325, A:Y326, A:E327, A:G328, A:T329, A:D330, A:A331, A:P332, A:C333, A:K334, A:I335, A:P336, A:Q340, A:D341, A:E342, A:K343, A:G344, A:V345, A:T346, A:Q347, A:N355, A:P356, A:I357, A:V358, A:T359, A:D360, A:K361, A:E362, A:K363, A:P364, A:V365, A:P372, A:G374, A:E375, A:S376, A:Y377, A:I378, A:V379, A:V380, A:G381, A:A382, A:G383, A:E384, A:K385, A:A386, A:L387, A:K388, A:L389, A:S390, A:W391, A:F392, A:K394	92	0.658
	4	A:G223, A:A224, A:S225, A:T226, A:P227, A:Q228	6	0.579
	5	A:F373, A:G395, A:S396, A:I398	4	0.514
DENV1NS1	1	A:D1, A:S2, A:G3, A:C4, A:V5, A:I6, A:N7, A:W8, A:K9, A:G10, A:R11, A:E12, A:L13, A:K14, A:C15, A:G16, A:S17, A:G18, A:I19, A:F20	20	0.919
	2	A:G159, A:F160, A:G161, A:I162, A:F163, A:T164	6	0.718
	3	A:D37, A:S38, A:K40, A:R41, A:S43, A:A44, A:G47, A:K48, A:A49, A:W50, A:E51, A:E52, A:G53, A:V54, A:C55, A:N73, A:E74, A:N76, A:H77, A:I78, A:L79, A:L80, A:E81, A:N82, A:D83, A:M84, A:K85, A:F86, A:T87, A:K101, A:K102, A:G105, A:P106, A:Q107, A:P108, A:M109, A:E110, A:H111, A:K112, A:Y113, A:S114, A:W115, A:K116, A:S117, A:W118, A:G119, A:K120, A:A121, A:K122, A:I123, A:I124, A:G125, A:A126, A:D127, A:V128, A:Q129, A:N130, A:T131, A:T132, A:P138, A:N139, A:T140, A:P141, A:E142, A:C143, A:P144, A:D146, A:Q147	68	0.714
	4	A:K189, A:D190, A:S191, A:K192, A:K206, A:N207, A:E208, A:T209, A:W210, A:K211, A:P226, A:K227, A:T230, A:W232, A:S233, A:N234, A:G235, A:V236, A:L237, A:S239, A:E240, A:I251, A:S252, A:Q253, A:H254, A:Y256, A:R257, A:P258, A:G259, A:Y260, A:F261, A:T262, A:D278, A:L279, A:C280, A:E281, A:G282, A:T283, A:T284, A:V285, A:V286, A:V287, A:D288, A:E289, A:H290, A:C291, A:G292, A:N293, A:P296, A:L298, A:R299, A:T300, A:T301, A:T302, A:V303, A:T304, A:G305, A:K306, A:I307, A:I308, A:H309, A:E310, A:W311, A:C312, A:C313, A:R314, A:S315, A:C316, A:T317, A:L318, A:P319, A:P320, A:D327, A:I335, A:R336, A:P337, A:V338, A:K339, A:D340, A:K341, A:E342, A:E343, A:N344, A:L345, A:V346, A:K347, A:S348	87	0.675
	5	A:A352, A:G353, A:S354, A:G355	4	0.569

Table 4.5- B cell conformational epitopes of DENV1 Envelope and NS1 protein predicted by ElliPro.

4.3.3.2 B cell conformational epitope prediction of DENV2

B cell conformational epitopes of DENV2 E and NS1 proteins are given in the following table 4.6.

Protein	no	Residues	No of residues	score
DENV2 Envelope	1	A:L425, A:G426, A:G427, A:V428, A:F429, A:T430, A:S431, A:I432, A:G433, A:K434, A:A435, A:L436, A:H437, A:Q438, A:V439, A:F440, A:G441, A:A442, A:I443, A:Y444, A:G445, A:A446, A:A447, A:F448, A:S449, A:G450, A:V451, A:S452, A:W453, A:T454, A:M455, A:K456, A:I457, A:L458, A:I459, A:G460, A:V461, A:I462, A:I463, A:T464, A:W465, A:I466, A:G467, A:M468, A:N469, A:S470, A:R471, A:S472, A:T473, A:S474, A:L475, A:S476, A:V477, A:S478, A:L479, A:V480, A:L481, A:V482, A:G483, A:I484, A:V485, A:T486, A:L487, A:Y488, A:L489, A:G490, A:V491, A:M492, A:V493, A:Q494	70	0.821
	2	A:K64, A:L65, A:T66, A:N67, A:T68, A:T69, A:T70, A:D71, A:S72, A:R73, A:C74, A:P75, A:T76, A:Q77, A:G78, A:E79, A:P80, A:T81, A:L82, A:N83, A:E84, A:E85, A:D87, A:K88, A:R89, A:F90, A:V91, A:C92, A:K93, A:H94, A:S95, A:M96, A:V97, A:D98, A:R99, A:G100, A:W101, A:G102, A:N103, A:G104, A:C105, A:G106, A:L107, A:F108, A:G109, A:K110, A:G111, A:G112, A:I113, A:V114, A:T115, A:C116, A:A117, A:M118, A:T120, A:A224, A:D225, A:T226, A:Q227, A:G228, A:S229, A:N230, A:W231, A:V238, A:T239, A:F240, A:K241, A:N242, A:P243, A:H244, A:A245, A:K246, A:K247, A:Q248, A:D249, A:V250, A:V251, A:V252, A:L253, A:G254, A:S255, A:Q256	82	0.764
	3	A:S19, A:P39, A:S145, A:G146, A:E147, A:E148, A:H149, A:D154, A:T155, A:G156, A:K157, A:H158, A:E174, A:L175, A:T176, A:G177, A:Y178, A:G179, A:T180, A:R288, A:D290, A:K291, A:L292, A:Q293, A:L294, A:K295, A:G296, A:M297, A:S298, A:Y299, A:S300, A:M301, A:C302, A:T303, A:G304, A:K305, A:F306, A:K307, A:S19, A:P39, A:S145, A:G146, A:E147, A:E148, A:H149, A:A150, A:E172, A:E174, A:L175, A:T176, A:G177, A:Y178, A:G179, A:T180, A:R288, A:D290, A:K291, A:L292, A:Q293, A:L294, A:K295, A:G296, A:M297, A:S298, A:Y299, A:S300, A:M301, A:C302, A:T303, A:G304, A:K305, A:F306, A:K307, A:V308, A:V309, A:K310, A:E311, A:I312, A:R323, A:V324, A:Q325, A:Y326, A:E327, A:G328, A:D329, A:G330, A:S331, A:P332, A:C333, A:K334, A:T335, A:P336, A:M340, A:D341, A:L342, A:E343, A:K344, A:R345, A:H346, A:V347, A:N355, A:P356, A:I357, A:V358, A:T359, A:E360, A:K361, A:D362, A:S363, A:P364, A:V365, A:P372, A:G374, A:D375, A:S376, A:Y377, A:I378, A:I379, A:I380, A:G381, A:V382, A:E383, A:P384, A:G385, A:Q386, A:L387, A:K388, A:L389, A:D390, A:W391, A:F392, A:K394	94	0.65
	4	A:G156, A:K157, A:H158	3	0.525
DENV2 NS1	1	A:D1, A:S2, A:G3, A:C4, A:V5, A:V6, A:S7, A:W8, A:K9, A:N10, A:K11, A:E12, A:L13, A:K14, A:C15, A:G16, A:S17, A:G18, A:I19, A:F20	20	0.929
	2	A:G159, A:F160, A:G161, A:V162, A:F163, A:T164	6	0.738
	3	A:E37, A:S38, A:S40, A:K41, A:A43, A:S44, A:Q47, A:K48, A:A49, A:H50, A:E51, A:E52, A:G53, A:I54, A:C55, A:P73, A:E74, A:N76, A:H77, A:I78, A:L79, A:S80, A:E81, A:N82, A:E83, A:V84, A:K85, A:L86, A:T87, A:M89, A:K101, A:S103, A:L104, A:Q105, A:P106, A:Q107, A:P108, A:T109, A:E110, A:L111, A:K112, A:Y113, A:S114, A:W115, A:K116, A:T117, A:W118, A:G119, A:K120, A:A121, A:K122, A:M123, A:L124, A:S125, A:T126, A:E127, A:S128, A:H129, A:N130, A:Q131, A:T132, A:E139, A:T140, A:A141, A:E142, A:C143, A:P144, A:T146, A:N147	69	0.703
	4	A:P226, A:K227, A:T230, A:S233, A:N234, A:G235, A:V236, A:L237, A:S239, A:E240, A:V251, A:Y256, A:R257, A:P258, A:G259, A:Y260, A:H261, A:T262, A:A265, A:D278, A:F279, A:C280, A:E281, A:G282, A:T283, A:T284, A:V285, A:V286, A:V287, A:T288, A:E289, A:D290, A:C291, A:G292, A:N293, A:R294, A:G295, A:P296, A:S297, A:L298, A:R299, A:T300, A:T301, A:T302, A:A303, A:S304, A:G305, A:K306, A:L307, A:I308, A:T309, A:E310, A:W311, A:C312, A:C313, A:R314, A:S315, A:C316, A:T317, A:L318, A:P319, A:P320, A:D327, A:M333, A:E334, A:I335, A:R336, A:P337, A:L338, A:K339, A:E340, A:K341, A:E342, A:E343, A:N344, A:L345, A:V346, A:N347, A:S348	79	0.653
	5	A:K189, A:D190, A:N191, A:A205, A:L206, A:N207, A:D208, A:T209, A:W210, A:L231, A:W232, A:S252, A:Q253, A:H254	14	0.639
	6	A:T351, A:A352, A:G353, A:H354, A:G355	5	0.539

Table 4.6- B cell conformational epitopes of DENV2 Envelope and NS1 protein predicted by ElliPro.

4.3.3.3 B cell conformational epitope prediction of DENV3

B cell conformational epitopes of DENV3 E and NS1 proteins are given in the following table 4.7.

Protein	No	Residues	No of residues	Score
DENV3 Envelope	1	A:G439, A:S440, A:A441, A:Y442, A:T443, A:A444, A:L445, A:F446, A:S447, A:G448, A:V449, A:S450, A:W451, A:I452, A:M453, A:K454, A:I455, A:G456, A:I457, A:G458, A:V459, A:L460, A:L461, A:T462, A:W463, A:I464, A:G465, A:L466, A:N467, A:S468, A:K469, A:N470, A:T471, A:S472, A:M473, A:S474, A:F475, A:S476, A:C477, A:I478, A:A479, A:I480, A:G481, A:I482, A:I483, A:T484, A:L485, A:Y486, A:L487, A:G488, A:V489, A:V490, A:V491, A:Q492, A:A493, A:D494, A:M495	57	0.851
	2	A:E62, A:G63, A:K64, A:I65, A:T66, A:N67, A:I68, A:T69, A:T70, A:D71, A:S72, A:R73, A:C74, A:P75, A:T76, A:Q77, A:G78, A:E79, A:A80, A:I81, A:L82, A:P83, A:E84, A:E85, A:D87, A:Q88, A:N89, A:Y90, A:V91, A:C92, A:K93, A:H94, A:T95, A:Y96, A:V97, A:D98, A:R99, A:G100, A:W101, A:G102, A:N103, A:G104, A:C105, A:G106, A:L107, A:F108, A:G109, A:K110, A:G111, A:S112, A:L113, A:V114, A:T115, A:C116, A:A117, A:K118, A:Q120, A:L122, A:G221, A:A222, A:T223, A:T224, A:E225, A:T226, A:P227, A:T228, A:W229, A:V236, A:T237, A:F238, A:K239, A:N240, A:A241, A:H242, A:A243, A:K244, A:K245, A:Q246, A:E247, A:V248, A:V249, A:V250, A:L251, A:G252, A:S253, A:Q254	86	0.752
	3	A:S429, A:L430, A:G431, A:K432, A:M433, A:V434, A:H435, A:Q436, A:I437, A:F438	10	0.728
	4	A:P39, A:T145, A:G146, A:D147, A:Q148, A:H149, A:Q150, A:V151, A:P174, A:E175, A:Y176, A:L292, A:K293, A:G294, A:M295, A:S296, A:Y297, A:A298, A:M299, A:C300, A:L301, A:N302, A:T303, A:F304, A:V305, A:L306, A:K307, A:K308, A:E309, A:V310, A:S311, A:E312, A:I320, A:K321, A:V322, A:E323, A:Y324, A:K325, A:G326, A:E327, A:D328, A:A329, A:P330, A:C331, A:K332, A:I333, A:P334, A:E338, A:D339, A:G340, A:Q341, A:G342, A:K343, A:A344, A:H345, A:N353, A:P354, A:V355, A:V356, A:T357, A:K358, A:K359, A:E360, A:E361, A:P362, A:V363, A:P370, A:G372, A:E373, A:S374, A:N375, A:I376, A:V377, A:I378, A:G379, A:I380, A:G381, A:D382, A:K383, A:A384, A:L385, A:K386, A:I387, A:N388, A:Y390, A:K392	86	0.662
	5	A:T19, A:E170, A:I172, A:G177, A:T178, A:D288, A:K289, A:L290	8	0.559
DENV3 NS1	1	A:G1, A:C2, A:V3, A:I4, A:N5, A:W6, A:K7, A:G8, A:K9, A:E10, A:L11, A:K12, A:C13, A:G14, A:S15, A:G16, A:I17, A:F18, A:V19, A:T20	20	0.885
	2	A:G157, A:F158, A:G159, A:V160, A:F161, A:T162	6	0.738
	3	A:D35, A:S36, A:P37, A:K38, A:R39, A:A41, A:T42, A:A43, A:A45, A:G46, A:A47, A:W48, A:E49, A:N50, A:G51, A:V52, A:C53, A:N71, A:E72, A:N74, A:Y75, A:I76, A:L77, A:W78, A:E79, A:N80, A:N81, A:I82, A:K83, A:L84, A:T85, A:G98, A:K99, A:R100, A:T101, A:L102, A:T103, A:P104, A:Q105, A:P106, A:M107, A:E108, A:L109, A:K110, A:Y111, A:S112, A:W113, A:K114, A:T115, A:W116, A:G117, A:K118, A:A119, A:K120, A:I121, A:V122, A:T123, A:A124, A:E125, A:T126, A:Q127, A:N128, A:S129, A:S130, A:P136, A:S137, A:T138, A:P139, A:E140, A:C141, A:P142, A:A144, A:S145	73	0.697
	4	A:K187, A:D188, A:E189, A:R190, A:Q203, A:K204, A:N205, A:G206, A:S207, A:W208, A:P224, A:K225, A:T228, A:S231, A:N232, A:G233, A:V234, A:L235, A:S237, A:D238, A:M239, A:I249, A:S250, A:Q251, A:H252, A:H254, A:R255, A:P256, A:G257, A:Y258, A:H259, A:T260, A:A263, A:N276, A:Y277, A:C278, A:E279, A:G280, A:T281, A:T282, A:V283, A:V284, A:I285, A:S286, A:E287, A:N288, A:C289, A:G290, A:T291, A:R292, A:G293, A:P294, A:S295, A:L296, A:R297, A:T298, A:T299, A:T300, A:V301, A:S302, A:G303, A:K304, A:L305, A:I306, A:H307, A:E308, A:W309, A:C310, A:C311, A:R312, A:S313, A:C314, A:T315, A:L316, A:P317, A:P318, A:D325, A:G330, A:M331, A:E332, A:I333, A:R334, A:P335, A:I336, A:N337, A:E338, A:K339, A:E341, A:N342, A:M343, A:V344, A:K345, A:S346	93	0.648
	5	A:R170, A:E171, A:V172	3	0.508

Table 4.7- B cell conformational epitopes of DENV3 Envelope and NS1 protein predicted by ElliPro.

4.3.3.4 B cell conformational epitope prediction of DENV4

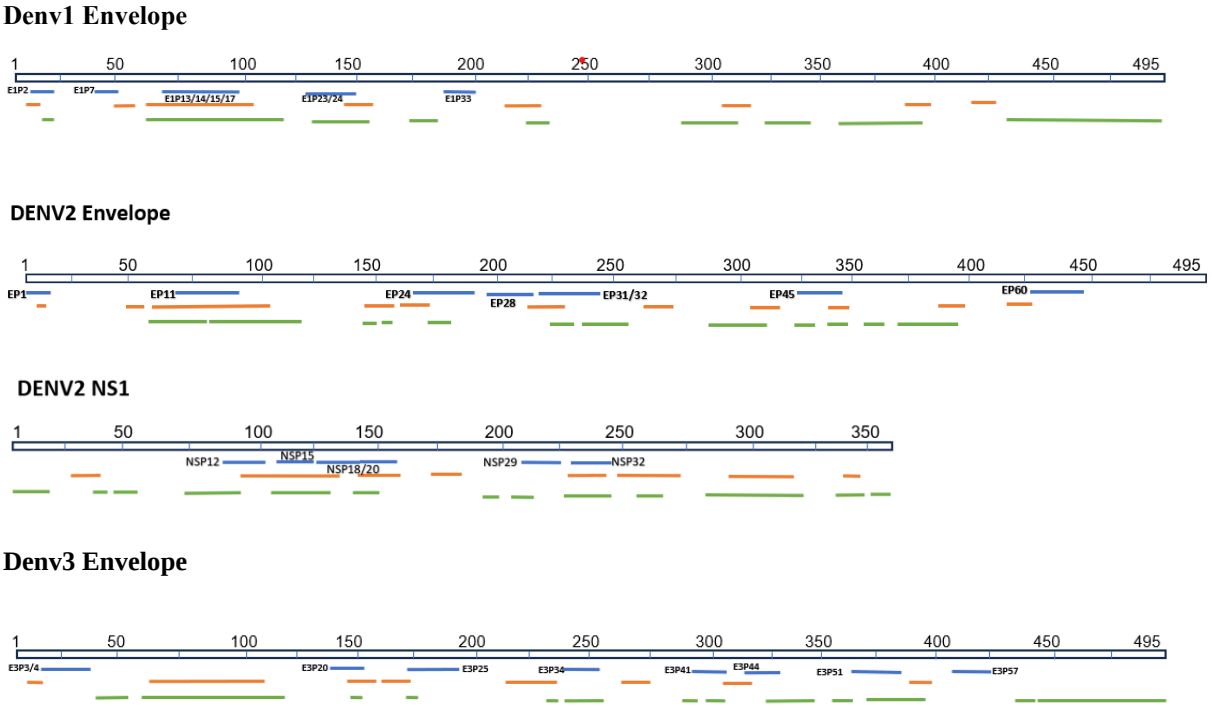
B cell conformational epitopes of DENV4 E and NS1 proteins are given in the following table 4.8.

Protein	No	Residues	No of res	Score
DENV4 Envelope	1	A:G426, A:L427, A:L428, A:S430, A:L431, A:A434, A:V435, A:H436, A:Q437, A:V438, A:F439, A:G440, A:S441, A:V442, A:Y443, A:T444, A:T445, A:M446, A:F447, A:G448, A:G449, A:V450, A:S451, A:W452, A:M453, A:V454, A:R455, A:I456, A:L457, A:I458, A:G459, A:F460, A:L461, A:V462, A:L463, A:W464, A:I465, A:G466, A:T467, A:N468, A:S469, A:R470, A:N471, A:T472, A:S473, A:M474, A:A475, A:M476, A:T477, A:C478, A:I479, A:A480, A:V481, A:G482, A:G483, A:I484, A:T485, A:L486, A:F487, A:L488, A:G489, A:F490, A:T491, A:V492, A:H493, A:A494	66	0.813
	2	A:E61, A:A62, A:S63, A:I64, A:S65, A:N66, A:I67, A:T68, A:T69, A:A70, A:T71, A:R72, A:C73, A:P74, A:T75, A:Q76, A:G77, A:E78, A:P79, A:Y80, A:L81, A:K82, A:E83, A:E84, A:D86, A:Q87, A:Q88, A:Y89, A:I90, A:C91, A:R92, A:R93, A:D94, A:V95, A:V96, A:D97, A:R98, A:G99, A:W100, A:G101, A:N102, A:G103, A:C104, A:G105, A:L106, A:F107, A:G108, A:K109, A:G110, A:G111, A:V112, A:V113, A:T114, A:C115, A:A116, A:K117, A:F118, A:S119, A:G222, A:A223, A:D224, A:T225, A:S226, A:E227, A:V228, A:H229, A:W230, A:N231, A:Y232, A:K233, A:E234, A:M236, A:V237, A:T238, A:F239, A:K240, A:V241, A:P242, A:H243, A:A244, A:K245, A:Q247, A:D248, A:V249, A:T250, A:V251, A:L252, A:G253, A:S254, A:Q255	90	0.739
	3	A:G36, A:P38, A:N144, A:G145, A:D146, A:T147, A:H148, A:P175, A:D176, A:Y177, A:I293, A:K294, A:G295, A:M296, A:S297, A:Y298, A:T299, A:M300, A:C301, A:S302, A:G303, A:K304, A:F305, A:S306, A:I307, A:D308, A:K309, A:E310, A:M311, A:V321, A:K322, A:V323, A:K324, A:Y325, A:E326, A:G327, A:A328, A:G329, A:A330, A:P331, A:C332, A:K333, A:V334, A:P335, A:E337, A:R339, A:D340, A:V341, A:N342, A:K343, A:E344, A:K345, A:V346, A:S352, A:T354, A:P355, A:F356, A:A357, A:E358, A:Y359, A:T360, A:N361, A:S362, A:V363, A:T364, A:P371, A:G373, A:D374, A:S375, A:Y376, A:I377, A:V378, A:I379, A:G380, A:V381, A:G382, A:D383, A:S384, A:A385, A:L386, A:T387, A:L388, A:H389, A:W390, A:F391, A:K393	86	0.662
	4	A:A18, A:E171, A:K173, A:G178, A:E179, A:R287, A:E289, A:K290, A:L291	9	0.589
	5	A:F372, A:G394, A:S395, A:I397	4	0.519
	6	A:S15, A:G16, A:G17	3	0.507
DENV4 NS1	1	A:R256, A:Q257, A:G258, A:Y259, A:G277, A:E278, A:C279, A:P280, A:G281, A:T282, A:T283, A:V284, A:T285, A:I286, A:Q287, A:E288, A:D289, A:C290, A:D291, A:H292, A:P295, A:L297, A:R298, A:T299, A:T300, A:T301, A:A302, A:S303, A:G304, A:K305, A:L306, A:V307, A:T308, A:Q309, A:W310, A:C311, A:C312, A:R313, A:S314, A:C315, A:T316, A:M317, A:P318, A:P319, A:D326, A:R335, A:P336, A:L337, A:S338, A:E339, A:K340, A:E341, A:E342, A:N343, A:M344, A:V345, A:K346, A:S347	58	0.728
	2	A:E36, A:S37, A:A39, A:R40, A:S43, A:L46, A:N47, A:A48, A:H49, A:K50, A:D51, A:G52, A:V53, A:C54, A:N72, A:E73, A:N75, A:Y76, A:V77, A:L78, A:W79, A:E80, A:G81, A:G82, A:H83, A:D84, A:L85, A:T86, A:K100, A:L103, A:A104, A:P105, A:P106, A:V107, A:N108, A:D109, A:L110, A:K111, A:Y112, A:S113, A:W114, A:K115, A:T116, A:W117, A:G118, A:K119, A:A120, A:K121, A:I122, A:F123, A:T124, A:P125, A:E126, A:A127, A:K128, A:N129, A:S130, A:T131, A:P137, A:D138, A:T139, A:S140, A:E141, A:C142, A:P143, A:E145, A:R146	67	0.726
	3	A:T1, A:G2, A:C3, A:A4, A:V5, A:S6, A:W7, A:S8, A:G9, A:K10, A:E11, A:K13, A:C14, A:G15, A:S16, A:G17, A:I18, A:D22, A:E155, A:D156, A:Y157, A:G158, A:F159, A:G160, A:M161, A:F162, A:T163, A:N165, A:K188, A:D189, A:Q190, A:K191, A:S204, A:K205, A:N206, A:Q207, A:T208, A:W209, A:Q210, A:E212, A:P225, A:K226, A:T229, A:L230, A:W231, A:S251, A:Q252, A:H253	48	0.677
	4	A:S232, A:N233, A:G234, A:V235, A:L236, A:S238, A:Q239, A:F250, A:Y255, A:A260, A:T261	11	0.572
	5	A:A351, A:G352, A:Q353, A:G354	4	0.563

Table 4.8- B cell conformational epitopes of DENV4 Envelope and NS1 protein predicted by ElliPro.

4.3.4 Computationally predicted epitope regions overlapped with Immunodominant peptides

Computationally predicted B cell epitopes were mapped on the peptides considered to contain immunodominant regions by serology**.** Identified immunodominant peptides of DENV Envelope and NS1 proteins were found to be overlapping with B cell linear and conformational epitope regions of the viral protein as shown schematically in Figure 4.4. Peptide E1P13-17 of DENV1 E had 100% amino acid sequence overlap with B cell linear and conformationa; epitopes Epitopes EP31, NSP12, NSP20 NSP32 had more than 10 amino acid sequence overlaps with B cell linear epitopes whereas EP24, EP32, EP45, EP60 and NSP18, NSP32 had more than or equal to 10 amino acid sequence overlaps with B cell conformational epitopes. NS3P5 of DENV3 had 14 amino acid sequence overlap with B cell linear epitope. Interestingly, EP11 (18) and NSP15(16) of DENV2 had 100% amino acid sequence overlap with B cell linear and conformational epitopes This finding confirms the epitope characteristics of the immunodominant peptides.



Denv3 NS1

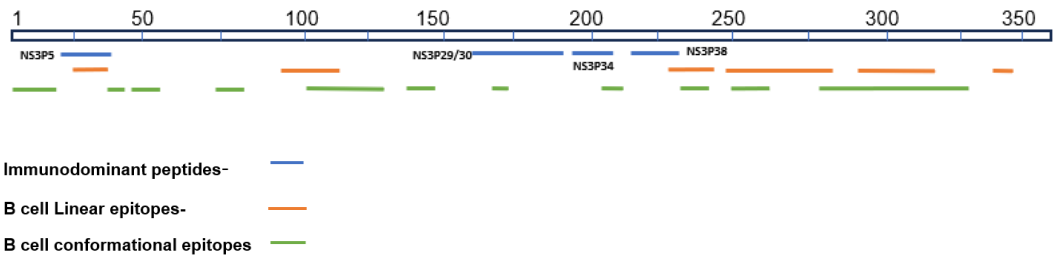


Figure 4.4: Schematic diagram of mapping immunodominant peptides and predicted B cell epitopes. Here Dengue Envelope and NS1 protein sequences are represented by a box of respective amino acid lengths. Immunodominant peptides identified by the Peptide array are represented by blue lines, B cell linear epitopes are represented by orange lines and B cell conformational epitopes are represented by green lines.

4.3.5 Conservancy of immunodominant peptides across consensus sequence

Conservancy across 50 consensus sequences at 100% sequence identity threshold showed the highest conservancy of 98% for EP32 and EP60 and 96% for EP1 for DENV2 peptides. Similarly, highest conservancy of 92% for the NSP32 peptide was seen among the DENV2 NS1 peptides. The conservancy of peptides in 200 pan Dengue sequences showed 62.5% for EP1 and 95.5% for NSP32 in 80% sequence match identity. All immunodominant peptides showed no sequence identity match with the J.E virus, thereby omitting the risk of cross-reactivity of these immunodominant peptides with the J.E virus. Similarly, all immunodominant peptides showed no sequence identity match with the Zika virus except for EP1. Peptides EP1(1.7575) and EP24(1.0170) showed the highest antigenic score among Envelope peptides and NSP15(0.8031) and NSP20 (0.8034) showed the highest antigenic score among NS1 peptides. The antigenicity score of immunodominant epitopes is given in each conservancy table (Table 4.9-4.11). Likewise conservancy analysis of DENV1 E peptides revealed E1P33 to be best conserved in serotype 1 with 92% conservancy and 74.5% conserved in pan serotype. E1P33 also showed no sequence similarity with either J.E or Zika sequences.

Sl.no	Epitope name	Sequence	length	Antigenicity score	Percent of protein sequence matches at identity 100%	Pan Dengue sequence matches at identity <= 80%	J.E sequence matches at identity <= 80%	Zika sequence matches at identity <= 80%
1	E1P2	GNRDFVEGLSGATWVDV	17	**0.3506**	**48.00% (24/50)**	**50.50% (101/200)**	**96.00% (48/50)**	**100.00% (50/50)**
2	E1P7	KDKPTLDIELLKTEVTN	17	**0.5199**	**64.00% (32/50)**	**25.50% (51/200)**	**0.00% (0/50)**	**0.00% (0/50)**
3	E1P13	SRCPTQGEATLVEEQDA	17	**0.7302**	**82.00% (41/50)**	**51.00% (102/200)**	**0.00% (0/50)**	**0.00% (0/50)**
4	E1P14	GEATLVEEQDANFVCRR	17	**1.2128**	**82.00% (41/50)**	**24.50% (49/200)**	**0.00% (0/50)**	**0.00% (0/50)**
5	E1P15	VEEQDANFVCRRTFVDR	17	**0.6689**	**82.00% (41/50)**	**24.50% (49/200)**	**0.00% (0/50)**	**0.00% (0/50)**
6	E1P17	RTFVDRGWGNGCGLFGK	17	0.0909	**92.00% (46/50)**	**100.00% (200/200)**	**100.00% (50/50)**	**92.00% (46/50)**
7	E1P23	GKIVQYENLKYSVIVTV	17	**0.8004**	**90.00% (45/50)**	**40.00% (80/200)**	**0.00% (0/50)**	**0.00% (0/50)**
8	E1P24	YENLKYSVIVTVHTGDQ	17	**0.7719**	**88.00% (44/50)**	**47.50% (95/200)**	**0.00% (0/50)**	**0.00% (0/50)**
9	E1P33	DCSPRTGLDFNEMVLLT	17	**0.6532**	**92.00% (46/50)**	**74.50% (149/200)**	**0.00% (0/50)**	**0.00% (0/50)**

Table 4.9: Conservancy of immunodominant peptides of DENV1 E across consensus sequence

Sl No.	Epitope name	Peptide sequence	Epitope length	Antigenicity score	Per cent of protein sequence matches at identity <= 100%	Pan Dengue sequence matches at identity <= 80%	J.E sequence matches at identity <= 80%	Zika sequence matches at identity <= 80%
1	EP1	MRCIGISNRDFVEGV	15	1.7575	**96.00% (48/50)**	**62.50% (125/200)**	**0.00% (0/50)**	100.00 % (50/50)
2	EP11	RCPTQGEPSLNEEQDKRF	18	0.6637	**80.00% (40/50)**	**25.00% (50/200)**	**0.00% (0/50)**	0.00% (0/50)

3	EP24	SITEAELTGYGTVTM	15	1.0170	**84.00% (42/50)**	**25.00% (50/200)**	**0.00% (0/50)**	0.00% (0/50)
4	EP28	LLQMEDKAWLVHRQWFL	17	0.7322	**38.00% (19/50)**	**25.00% (50/200)**	**0.00% (0/50)**	0.00% (0/50)
5	EP31	PGADTQGSNWIQKETLV	17	0.2179	**90.00% (45/50)**	**25.00% (50/200)**	**0.00% (0/50)**	0.00% (0/50)
6	EP32	SNWIQKETLVTFKNPHAK	18	0.3084	**98.00% (49/50)**	**25.00% (50/200)**	**0.00% (0/50)**	0.00% (0/50)
7	EP45	VQYEGDGSPCKIPFEIM	17	0.268	**70.00% (35/50)**	**25.00% (50/200)**	**0.00% (0/50)**	0.00% (0/50)
8	EP60	VFTSIGKALHQVFGAIY	17	0.284	**98.00% (49/50)**	**25.50% (51/200)**	**0.00% (0/50)**	0.00% (0/50)
9	NSP12	KLTIMTGDIKGIMQAGKR	18	0.3611	**76.00% (38/50)**	**25.00% (50/200)**	**0.00% (0/50)**	0.00% (0/50)
10	NSP15	TELKYSWKTWGKAKML	16	0.8031	**74.00% (37/50)**	**49.50% (99/200)**	**0.00% (0/50)**	0.00% (0/50)
11	NSP18	TESHNQTFLIDGPETA	16	-0.087	**38.00% (19/50)**	**25.00% (50/200)**	**0.00% (0/50)**	0.00% (0/50)
12	NSP20	TAECPNTNRAWNSLEV	16	0.8034	**84.00% (42/50)**	**29.50% (59/200)**	**0.00% (0/50)**	0.00% (0/50)
13	NSP29	TWKIEKASFIEVKSCHW	17	0.7563	**56.00% (28/50)**	**25.00% (50/200)**	**0.00% (0/50)**	0.00% (0/50)
14	NSP32	SHTLWSNGVLESEMIIPK	18	-0.0299	**92.00% (46/50)**	**95.50% (191/200)**	**0.00% (0/50)**	0.00% (0/50)

Table 4.10: Conservancy of immunodominant peptides of DENV2 E and NS1 across consensus sequence

E3P34 peptide of DENV3 E also showed 96% intra serotype conservancy and 74.5% pan serotype conservancy and no sequence match identity was seen with J.E or Zika. For DENV3 NS1 peptides NS3P34 and NS3P38 showed 92% and 88% intra serotype conservance respectively. These two epitopes was also foundt o be higly conserved in all DENV serotypes with 97% sequence conservancy score.

Sl.no	Epitope name	Sequence	length	Percent of protein sequence matches at identity 100%	Pan Dengue sequence matches at identity <= 80%	J.E sequence matches at identity <= 80%	Zika sequence matches at identity <= 80%
1	E3P3	SGATWVDVVLEHGGCV	16	**96.00% (48/50)**	**75.00% (150/200)**	**100.00% (50/50)**	**0.00% (0/50)**
2	E3P4	DVVLEHGGCVTTMAKNK	17	**96.00% (48/50)**	**99.50% (199/200)**	**96.00% (48/50)**	**0.00% (0/50)**
3	E3P20	LKYTVIITVHTGDQHQV	17	**92.00% (46/50)**	**48.50% (97/200)**	**0.00% (0/50)**	**0.00% (0/50)**
4	E3P25	ILPEYGTLGLECSPRTGL	18	**86.00% (43/50)**	**25.00% (50/200)**	**0.00% (0/50)**	**0.00% (0/50)**
5	E3P34	LVTFKNAHAKKQEVVVL	17	**96.00% (48/50)**	**74.50% (149/200)**	**0.00% (0/50)**	**0.00% (0/50)**
6	E3P41	DKLELKGMSYAMCLNAFV	18	**6.00% (3/50)**	**25.00% (50/200)**	**0.00% (0/50)**	**0.00% (0/50)**
7	E3P44	EVSETQHGTILIKVEYK	17	**96.00% (48/50)**	**25.00% (50/200)**	**0.00% (0/50)**	**0.00% (0/50)**
8	E3P51	PVNIEAEPPFGESNIVI	17	**80.00% (40/50)**	**70.50% (141/200)**	**0.00% (0/50)**	**0.00% (0/50)**
9	E3P57	ARGARRMAILGDTAWDF	17	**100.00% (50/50)**	**100.00% (200/200)**	**100.00% (50/50)**	**0.00% (0/50)**
10	NS3P5	NEVHTWTEQYKFQAD	15	**98.00% (49/50)**	**50.00% (100/200)**	**0.00% (0/50)**	**0.00% (0/50)**
11	NS3P29	GVFTTNIWLKLREVYTQ	17	**64.00% (32/50)**	**48.50% (97/200)**	**0.00% (0/50)**	**0.00% (0/50)**
12	NS3P30	IWLKLREVYTQLCDHRL	17	**24.00% (12/50)**	**48.50% (97/200)**	**0.00% (0/50)**	**0.00% (0/50)**
13	NS3P34	DERAVHADMGYWIESQK	17	**92.00% (46/50)**	**99.00% (198/200)**	**0.00% (0/50)**	**0.00% (0/50)**
14	NS3P38	KASLIEVKTCTWPKSHT	17	**88.00% (44/50)**	**97.00% (194/200)**	**0.00% (0/50)**	**0.00% (0/50)**

Table 4.11: Conservancy of immunodominant peptides of DENV3 E and NS1 across consensus sequence

4.3.6 Surface localization of immunodominant peptides

Structural localization of immunodominant peptides was visualised by using PyMOL. All the identified immunodominant peptides of DENV1-3 E and NS1 proteins were present on the surface of proteins which justifies the affinity of serum antibodies binding more efficiently to these immunodominant epitopes.

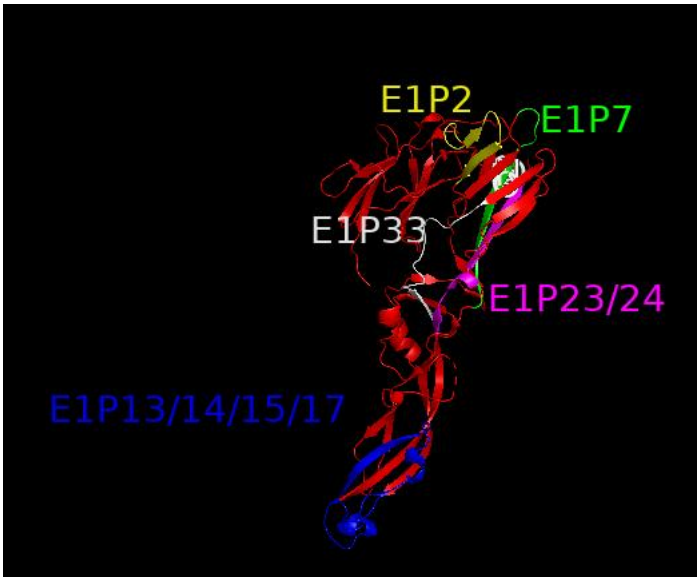
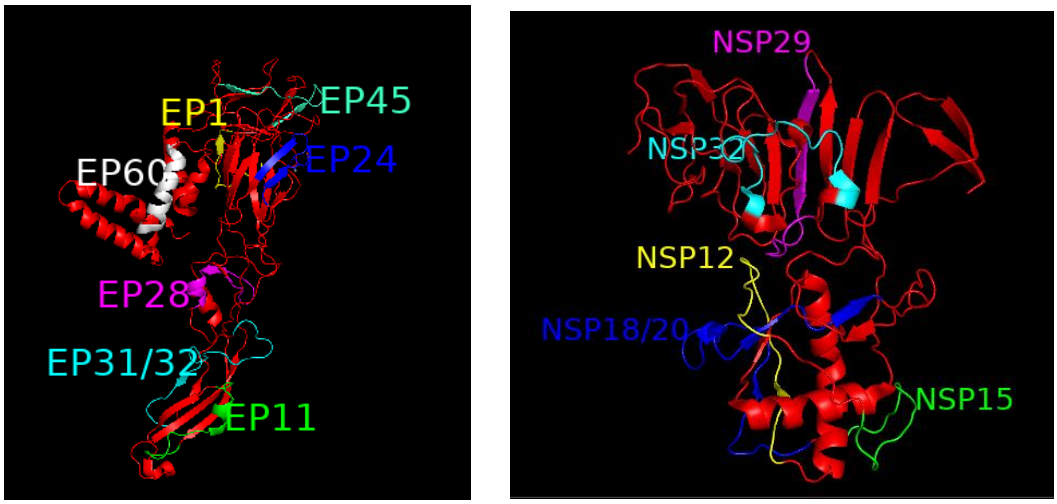


Figure 4.5: Surface localization of immunodominant peptides A) Envelope (3G7T) proteins of DENV1. Each peptide has been represented by different colours and the whole protein by red colour.



A **B**

Figure 4.6: Surface localization of immunodominant peptides A) Envelope (3J2P) and B) NS1(7WUR) proteins of DENV2. Each peptide has been represented by different colours and the whole protein by red colour.

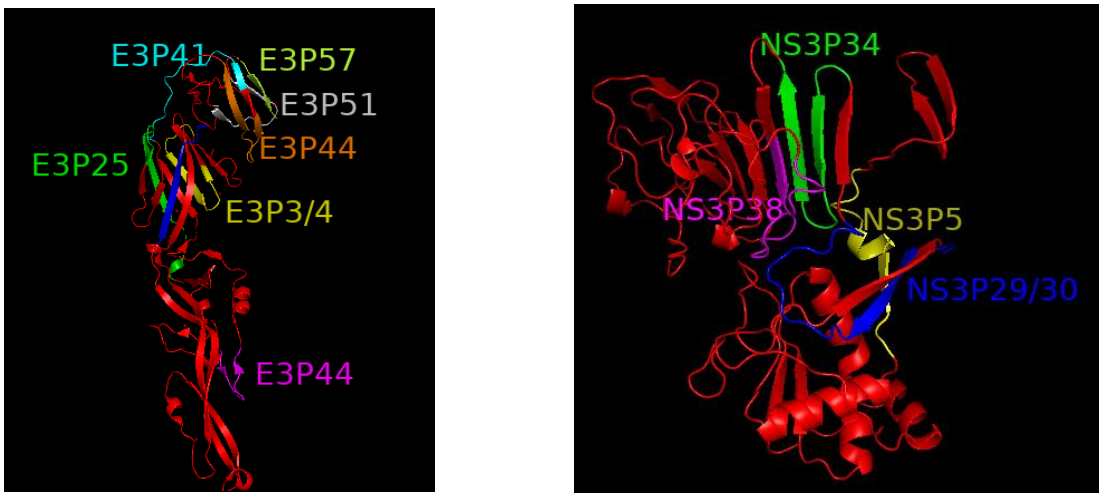


Figure 4.7: Surface localization of immunodominant peptides A) Envelope (7A3P) and B) NS1proteins of DENV3. Each peptide has been represented by different colours and the whole protein by red colour.

4.3.7 T cell epitope prediction of DENV E and NS1 proteins

8, 5, 8 and 5 potential MHC Class I T cell epitopes of DENV 1,DENV2, DENV3 and DENV4 envelope proteins were predicted. Epitopes in position 238-246 of DENV1 &2 position 236-244 of DENV3 and 238-247 of DENV4 share approximately 90% sequence similarity. This T cell epitope is conserved in all serotype.

Serotype	No of epitopes	start	end	sequence	Antigenic score (Vaxijen 2.0)
DENV1	8	424	432	SIGGVFTSV	0.4735
		200	208	TMKEKSWLV	1.2922
		238	**246**	**VTFKTAHAK**	**0.5252**
		286	295	RLKMDKLTLK	-0.1075
		212	220	WFLDLPLPW	0.3261
		276	284	TTIFAGHLK	0.3016
		386	377	ALKLSWFKK	0.6427
		50	58	VTNPAVLRK	-0.0574
DENV2	5	346	354	HVLGRLITV	-0.5400
		424	432	SLGGVFTSI	0.4141
		474	482	SLSVSLVLV	1.1662
		476	484	SVSLVLVGV	1.3768
		238	**246**	**VTFKNPHAK**	**0.5252**
DENV3	8	429	437	SLGKMVHQI	0.2960
		444	452	ALFSGVSWI	0.4518
		348	356	RLITANPVV	-0.2611
		106	114	GLFGKGSLV	0.8202
		422	430	SVGGVLNSL	0.2836
		350	358	ITANPVVTK	0.4101
		236	**244**	**VTFKNAHAK**	**0.5252**
		128	136	KVVQHENLK	0.2453
DENV4	5	200	208	KMKKKTWLV	0.8780
		165	173	TPRSPSVEV	
		238	**247**	**VTFKVPHAKR**	**0.5252**
		232	240	NYKERMVTF	0.4191
		212	220	WFLDLPLPW	0.3261

Table 4.12: Predicted T cell epitopes MHC Class I of DENV envelope protein

8, 8, 4 and 9 potential MHC Class I T cell epitopes of DENV 1,DENV2, DENV3 and DENV4 NS1 proteins were predicted.

Serotype	No of epitopes	start	end	sequence	Antigenic score (Vaxijen 2.0)
DENV1	8	223	231	CVWPKSHTL	0.2373
		170	178	KLRDSYTQV	0.3145
		344	352	NLVKSLVSA	-0.4519
		316	324	CTLPPLRFK	1.5936
		38	46	SPKRLSAAI	0.6164
		107	115	QPMEHKYSW	**0.5869**
		180	188	DPRLMSAAI	0.8342
		105	113	GPQPMEHKY	0.5426
DENV2	8	78	86	ILSENEVKL	**0.1095**
		344	352	NLVNSLVTA	**0.1095**
		41	49	KLASAIQKA	**0.2711**
		103	111	SLRPQPTEL	**1.7975**
		133	141	FLIDGPETA	**-0.5215**
		67	75	MWKQITPEL	**0.1255**
		219	227	EVKSCHWPK	**0.7862**
		107	115	QPTELKYSW	0.5869
		316	324	CTLPPLRYR	**1.4685**
DENV3	4	219	227	EVKTCTWPK	**-0.2455**
		243	251	IPKSLAGPI	-0.7191
		105	113	TPQPMELKY	1.4232
		107	115	QPMELKYSW	0.5869
DENV4	9	41	49	RLASAILNA	0.1447
		216	224	SLIEVKTCL	0.2605
		235	243	GVLESQMLI	0.5527
		103	111	ALAPPVNDL	**0.1959**
		78	86	VLWEGGHDL	**-0.4986**
		223	231	CLWPKTHTL	**0.6607**
		67	75	MWKQITNEL	**-0.6468**
		224	232	LWPKTHTLW	**0.4351**
		105	113	APPVNDLKY	0.9653

Table 4.13: Predicted T cell epitopes MHC Class I of DENV NS1 protein

7, 8, 8 and 7 potential MHC Class II T cell epitopes of DENV 1,DENV2, DENV3 and DENV4 envelope proteins were predicted. Epitopes in position 235-248,234-248,232-246 and 234-248 of DENV1, DENV2, DENV3 and DENV4 respectively share a sequence similarity and so this T cell epitope is conserved in all DENV serotypes.

Serotype	No of epitopes	start	end	sequence	Antigenecity
DENV1	7	235	248	DLLVTFKTAHAKKQ	0.7461
		462	476	LLTWLGLNSRSTSLS	1.4511
		204	218	KSWLVHKQWFLDLPL	0.1325
		131	145	QYENLKYSVIVTVHT	0.7394
		375	389	ESYIVVGAGEKALKL	0.8964
		12	26	VEGLSGATWVDVVLE	0.4516
		410	424	RRMAILGDTAWDFGS	1.2873
DENV2	8	462	476	IITWIGMNSRSTSLS	1.3007
		410	424	KRMAILGDTAWDFGS	1.2817
		133	147	ENLEYTIVITPHSGE	0.7681

		402	416	FETTMRGAKRMAILG	0.7672
		194	208	NEMVLLQMENKAWLV	0.8388
		204	218	KAWLVHRQWFLDLPL	0.1451
		234	248	KETLVTFKNPHAKKQ	0.3284
DENV3	8	410	424	MAILGDTAWDFGSVG	1.6418
		232	246	KELLVTFKNAHAKKQ	0.4869
		194	208	MILLTMKNKAWMVHR	1.1853
		438	452	FGSAYTALFSGVSWI	0.2201
		345	359	HNGRLITANPVVTKK	0.3544
		132	146	HENLKYTVIITVHTG	0.763
		75	89	PTQGEAILPEEQDQN	0.5109
		208	218	WMVHRQWFFDLPLPW	0.4639
DENV4	7	449	463	GGVSWMVRILIGFLV	0.5785
		348	362	VGRIISSTPFAEYTN	0.1252
		38	52	KPTLDFELIKTTAKE	0.7738
		194	208	NEMILMKMKKKTWLV	0.7604
		234	248	KERMVTFKVPHAKRQ	0.2025
		426	440	GGLLTSLGKAVHQVF	0.2321
		210	214	KQWFLDLPLPWAAGA	0.3030

Table 4.14- Envelope MHC Class II binding T cell epitopes

6, 5, 6 and 6 potential MHC Class II T cell epitopes of DENV 1,DENV2, DENV3 and DENV4 NS1 proteins were predicted. Epitopes in position 29-43 was predicted for all DENV serotypes share a sequence similarity and so this T cell epitope is conserved in all DENV serotypes. On the other hand epitopes in position 208-222 was predicted for DENV1, DENV3 and DENV4 serotypes.

Serotype	**No of epitopes**	**start**	**end**	**sequence**	**Antigenecity**
DENV1	6	208	222	ETWKLARASFIEVKT	0.3548
		73	87	NELNHILLENDMKFT	0.7525
		160	174	FGIFTTNIWLKLRDS	0.7821
		29	43	TEQYKFQADSPKRLS	-0.08
		341	355	KEENLVKSLVSAGSG	0.1458
		98	112	AQGKKMIGPQPMEHK	0.4147
DENV2	5	129	143	HNQTFLIDGPETAEC	-0.128
		29	43	TEQYKFQPESPSKLA	0.7110
		146	160	TNRAWNSLEVEDYGF	1.1521
		226	240	PKSHTLWSNGVLESE	0.1306
		160	174	FGIFTTNIWLKLRDS	0.7821
DENV3	6	208	222	ETWKLARASFIEVKT	0.3548
		74	88	ELNYILWENNIKLTV	0.7366
		29	43	TEQYKFQADSPKRLA	-0.08
		112	126	KYSWKTWGKAKIVTA	0.2672
		341	355	KEENMVKSLASAGSG	0.2478
		274	288	ELDFNYCEGTTVVIS	0.6883

DENV4	6	341	355	KEENMVKSLASAGSG	0.2478
		15	29	CGSGIFVIDNVHTWT	-0.463
		208	222	QTWQIEKASLIEVKT	0.3548
		64	78	ENIMWKQITNELNYV	0.5187
		128	142	AKNSTFLIDGPDTSE	0.1410
		29	43	TEQYKFQPESPARLA	-0.08

Table 4.15- DENV NS1 MHC Class II binding T cell epitopes

4.4 Discussion

Immunoinformatics approaches enabled by advances in genomics, proteomics, and epitope mapping techniques are being widely applied in vaccinology and in diagnostics. However, due to their location on virion, computationally predicted linear B cell epitopes may have issues with antibody accessibility; therefore, the true immunogenicity of these epitopes must be assessed against antibodies that have naturally developed against the virus during infections. In our present study, we have mapped computationally predicted epitopes on immunodominant peptides determined by serology using a peptide array of DENV Envelope and NS1 proteins in DENV positive and negative sera to identify potential epitopes.

Epitope-based recombinant peptides have gained the attention of many researchers worldwide especially during and after the COVID-19 pandemic. B cell epitopes of SARS-CoV2 were determined computationally and validated for serological diagnosis by ELISA in COVID-19 serum achieving a sensitivity of 94% and a specificity of 97.2% [21]. A similar approach has also been used in Flavivirus epitope mapping by Victor Hugo Aquino et. al where they have identified linear epitope hotspots of E and NS1 proteins of Dengue and Zika viruses from serum samples of Dengue and Zika-positive patients[22]. More recently, an ELISA-based multi-epitope antigen derived from a fragment of NS1 protein of four dengue serotypes yielded 77.42% sensitivity and 88.57% specificity[23]. These findings highlight the use of recombinant peptides as a new tool for serological diagnosis of diseases.

In our investigation, sera obtained from DENV-infected patient exhibited a significant positive reaction to B cell linear epitopes, suggesting that these epitopes were accessible to antibodies. In an earlier study[2017] peptide region 72-88 of Envelope protein corresponding to our EP11 peptide was reported to be immunogenic and highly responsive to sera and was seen to be conserved[24] This region is located in the highly

conserved fusion loop (FL) (97–111 aa) and bc loop (73–79 aa) of the E protein's DII domain, which are important in fusion with the host cell membrane and enable the virion's nucleocapsid to invade the host cell. Given that EP11 falls in Domain II of E protein, which is involved in fusion, this epitope is expected to be an important target of host defense and thereby potential target of diagnosis and therapeutics. R. Anvikar *et al* [2020] also identified 5 best immunoreactive epitopes for each of E and NS1 proteins[25]. Comparison of sequences of epitopes identified in the present study with the immunoreactive epitopes revealed sequence correspondence between the two studies confirming the immunogenicity and their potential for use in diagnosis.

EP1 of this study had the highest conservancy in serotype 2 as well as in pan Dengue sequences. Further, at 80% sequence identity analysis of this particular peptide, this sequence was found to be partially conserved in Zika Virus as well. This peptide region represents the start sequences of Envelope protein and given that DENV and ZIKV share 55.6% amino acid sequence identity, there is a probability of epitope sharing and EP1 could be exploited for the development of DENV-ZIKV duo diagnostics. E1P33 to be best conserved in intra serotype with 92% conservancy and 74.5% conserved in pan serotype. E1P33 also showed no sequence similarity with either J.E or Zika sequences. E3P34 and NS3P34 also showed greater than 90% pan serotype conservancy.These peptides could be a promising candidate for diagnosis of DENV infection.

Epitopes EP32 and NSP32 though predicted to be non-antigenic, their overlapping sequence with T cell epitope is worth mentioning, suggesting their potential to elicit T cell response [26]. Prediction of potential and conserved T cell epitopes adds up the value of the study. The immunodominant peptides identified in the present study were seen to localize on the surface of the E and NS1 proteins respectively as confirmed by PyMOL software. Given their antigenicity, their potential as B cell epitopes on native protein is indicated.

Four NS1 immunodominant peptides, NSP12, NSP15, NSP18, and NSP20 fall within the wing domain which is an epitope-rich domain (residues 31-181) of NS1 protein. A recent study revealed that intertwined loop region (108-130) of wing domains of NS1 interact with the membrane[27]. Interestingly this region overlapped with peptide NSP15 identified in our study. Also, the amino acid sequence of NSP15 had 100% overlap with B cell linear and conformational epitopes. This discovery validates the immunodominant

peptides' epitope properties and could be a suitable therapeutic target.

The determination of epitopes based on correspondence between serology and computational epitope mapping followed by evaluating antibody response using sera from people with well-defined DENV infections marks the strength of our study.

4.5 References

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