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Identification of dengue virus proteome B-cell epitopes using an immunoinformatic approach

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ABSTRACT

Dengue is a disease caused by one of DENV1, DENV2, DENV3, and DENV4 serotypes. There is no successful vaccine available to control all serotypes of dengue virus. Therefore, we are discovering new prevention measures using immunoinformatics strategies to establish an epitope based subunit vaccine that can produce different immune responses within the host. The prediction and subsequent discovery of B-cell epitopes using *in silico* techniques will improve the authors' knowledge in pathogenesis of diseases and the development of better vaccines. In present work, for the analysis of serotype DENV2 proteomes, three separate prediction approaches, such as ABCpred, BCpred, and AAP method were used, which leads to the prediction of 1458 B cell epitopes. Antigenicity, allergicity, and toxicity were analyzed for selected 66 epitopes. Eight antigenic epitopes were predicted among 27 consensus epitopes. The IEDB conservancy tool evaluated six of them and found to be more than 75% conservancy. The research using the IEDB conservancy tool suggests that six possible novel epitopes as VEPGQLKLSWFKKGSSIGQM, TELKYSWKTWGK, NDWDFVVTTDIS, AKKQDVVLGSQEGAM, EIAETQHGTIVVRVQYEGDG, and DGITVIDLDPIPYDPK is expected to be unreported peptides.

INTRODUCTION

The dengue virus belonging to *Flaviridae* genus of the Flaviridae family is a common disease that affects about 400 million humans per year worldwide (Guzman *et al*., 2010; Kuno *et al*., 1998; Westaway *et al*., 1985). In 2020, India reported 16,439 cases, including 12 deaths, while the Lao PDR reported 6,015 cases of dengue and 11 deaths in 2020. 78,303 cases and 127 deaths have been reported in Malaysia (ECDC, 2020). 494 cases were reported from Bangladesh and 9,108 cases and 14 deaths from Cambodia, respectively. Nepal has reported 315 cases of dengue and Pakistan has reported a total of 743 cases of dengue by 2020 (ECDC, 2020). A record dengue fever epidemic in Latin America was reported in 2019, with over 2.7 million cases and 1,206 deaths during 10 months of 2019 (WHO, 2019). The genome of the Dengue virus consists of 10,696 nucleotides, of which 10,173 nucleotides encode 3,391 amino acids for a single open reading frame (Osatomi and Sumiyoshi, 1990). The viral genome of DEN encodes structural proteins as polyprotein, 2K peptide, Membrane glycoprotein, Envelope protein, Membrane Envelope protein, Anchored capsid protein, Capsid protein, and non-structural protein NS1, NS2a, NS2b, NS3, NS4A, NS4B, and NS5. A variety of human disorders are caused by dengue infection, ranging from asymptomatic, diarrhea, rash, knee pain and other mild symptoms to dengue hemorrhagic fever, and dengue shock syndrome. An important role is played by the immune system in embodying defensive responses as the first barrier to defense (Sai *et al*., 2013). During the acute phase of the febrile condition, a drop in platelet counts also occurs. After defervescence, most patients are resurrected, but extreme complications arise at this point as the fever subsides in a few patients, and this can potentially be lethal.

Dengvaxia, the world's first authorized dengue vaccine, is a tetravalent live attenuated dengue vaccine officially approved in 19 nations (Scott, 2016; WHO, 2017). Its overall effectiveness against DENV was low, with approximately 50% against DENV1 and 39% against DENV2 in particular. However, recent clinical trials have found that CYDTDV vaccination has induced a high risk of hospitalization for children under the age of nine (Hadinegoro

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et al., 2015). Clinical trials of Dengvaxia have shown that the DENV2 strain is not very successful. Present vaccination testing findings suggest that multiple people have had severe potential allergic responses, such as allergies, headache, and urticaria. The outcomes of clinical trials of Dengvaxia demonstrate adverse consequences for a pregnant woman resulting in miscarriage, stillbirth, elective termination, and uterine mortality.

Lymphocytes are one group, and lymphocytes include T, B, and NK cells. Cell activation, antigen detection, and signal transduction are the most important external structures of B-cells responsible for (Sathe and Cusick, 2020) the antigen receptor is the functionally part of the cell surface multi molecular protein complexes (Justiz Vaillant *et al*., 2020). The sequencing of the repertoire of B cells and the growing structural characterization of protective antigens and epitopes currently provide molecular and mechanical expertise to guide the production of new vaccines that were previously impossible. A transmembrane receptor that reaches into the cytoplasm is the B-cell anti gen receptor. In transmitting the signals and triggering B cells, these tails are ineffective (Tanaka and Baba, 2020). The B cell receptor is a complex of multi molecular proteins non covalently bound to other proteins. B cell epitopes are intended for the production of diagnostic reagents and vaccines, not just for pathogenesis and immunological research (Jiang *et al*., 2010). Consequently, because of its potential to be bound by antibodies, the B cell epitope is antigenic (detected by immune system, potentially). If it is also capable of activating the production of such antibodies (e.g., by binding them to B cell surface immunoglobulins), it is immunoglobulinic. The characteristics of being antigenic and immunogenic are also antigenic and immunogenic.

Predicting linear B-cell epitopes with high accuracy is of key importance for epitope-based immunotherapy. For predicting B cell epitopes, several bioinformatics servers and algorithms are available. Prediction algorithms for B cell epitopes rely on linear epitopes since it is known that linear epitopes can evoke an antibody response that can interact with the parental antigen (Saha and Raghava, 2007a, 2007b). B-cell epitope can be more immunogenic than another if both are present on the same molecule and in particular, if they overlap physically with each other so that, compared to the other, the more immunogenic one thus described as immunodominant will induce the production of antibodies on its own while effectively suppressing productivity.

MATERIALS AND METHODS

Collection of source data

Resource gene database of structural protein amino acid sequence polyprotein, 2K peptide, membrane glycoprotein, protein envelope, membrane protein envelope, capsid protein anchor, capsid protein and nonstructural protein NS1, NS2a, NS2b, NS3, NS4A, NS4B, and NS5 Dengue virus (https:/www.viprbrc.org/brc/vipr proteinserch.spg). For the prediction of B cell epitopes, proteomes of DV-2 serotypes were evaluated.

Prediction of linear B-cell epitopes

Using multiple epitope properties, three separate methods were used to predict B cell epitopes.

ABCpred (http://www.imtech.res.in/raghava/abcpred/) and BCPREDS (http://ailab.ist.psu.edu/bcpred/predict.html), were used to predict potential 12, 16, and 20 mer B-cell epitopes from dengue virus 2 serotype proteins. ABCPred is a tool based on a neural network for predicting continuous B-cell epitopes by means of a fixed length pattern (Saha and Raghava, 2006). The ABCPred dataset includes data from virus epitopes contained in the BciPep database with 65.9% prediction accuracy. In both the B cell epitope prediction servers, fixed duration patterns are common. BCPREDS comprises two fixed length methods BCPred and AAP methods. For B-cell epitope prediction, BCPred and AAP methods were chosen in this analysis. The default parameter given by the servers was used to calculate the B-cell epitope prediction.

Antigenicity prediction

Antigenicity is a vaccine property that specifies whether an antigen or epitope will react with antibodies or not and induces the immune system from subsequent challenge by DENV to establish a protective mechanism. Using VaxiJen (http://www.ddg pharmfac.net/vaxijen/VaxiJen /VaxiJen.html) to ascertain the existence of antigen in its sequence, the proteins acquired undergo antigenicity prediction. The peptide sequence with a VaxiJen value of 0.4 is anticipated to have antigenicity properties above the viral threshold level. The expected B-cell epitopes should be theoretically antigenic, so that lymphocytes can elcitate optimum immune response upon parental antigen presentation.

Predictions the allergenicity of protein

Allergen FP (http://ddg-pharmfac.net/Allergen FP) was used to predict allergenicity of proteins. Non allergenic protein picked on a similarity index basis.

Toxicity prediction

The anticipated putative candidates were unable to cause any adverse effects on humans during administration. The toxic nature of the epitopes anticipated was then analyzed using a web-based server Toxinpred (http://www.imtech.res.in/raghava/toxinpred/design.php).

Consensus epitope prediction

For the preparation of Dengue Virus vaccines, the prediction between or within current serotypes of common epitopes can be used. The results of the predicted dengue virus 2 serotype epitopes (12, 16, and 20 mer) were compared to each other and the typical peptides were found to be consensus epitopes. The key explanation for the use of the consensus epitope strategy was to classify putative candidates with a higher likelihood of Dengue Virus immune response.

Conservancy analysis

Conservancy study is used to determine the degree of distribution of the epitope in a homologous protein sample in the *in silico* vaccinology technique. We used the epitope conservancy research method (http:/tools.iedb.org/conservancy/) at the IEDB (Immune Epitope DataBase) for the prediction of the conservancy trend of the desired epitopes (Bui *et al*., 2007). We used a method to calculate the variability of epitopes within a given range of protein sequences to aid with the optimal degree of conservation in the collection of epitopes.

RESULTS

B-cell epitopes prediction

The DENV2 proteome generated 1458 B-cell epitopes in total. The ABCpred server predicted the largest number of B-cell epitopes 873, followed by 330 and 255 epitopes predicted by the BCpred and AAP methods of the BCPREDS server as shown in Figure 1. Out of 1458 epitopes examined by fixed length pattern (12, 16, and 20 mer), 681 epitopes (46.70%) were 12 mer in length, 330 (22.63%), 16 mer and 447 (30.65%) 20 mer length, respectively. Less than one epitope score predicted by every server was discarded. On the basis of score value 1 or more, a total of 66 epitopes were chosen for further analysis.

Antigenicity prediction

VaxiJen 2.0 was used to estimate the vaccine construction antigenicity for 66 epitopes and resulted in the antigenic score of more than 0.8 chosen for further study of all 66 epitopes as shown in Table 1. BCpred was chosen among the largest numbers of antigenic epitopes (55), followed by AAppred (11) and no epitopes were selected from ABCpred. The epitopes expected by each approach displayed different degrees of antigenicity: 0.8–1.3 for BCpred, 0.8–1.7. For AApred, 83.33% (55 out of 66) of the epitopes in the analysis showed an antigenic score greater than 0.8 and 16.66% of the BCpred analysis showed an antigenic score greater than 0.8 (11 out of 36) epitopes.

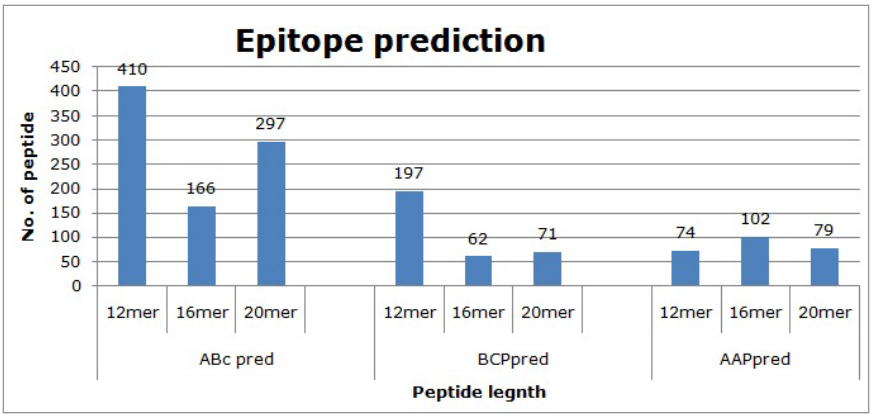

Figure 1. Prediction of the epitope by methods of ABCpred, BCpred, and AAP. The maximum epitope numbers were found in the study of ABCpred.

Table 1. Predictions of antigenicity, allergenicity and toxicity index of epitopes.

Tool	Protein name	Start position	Epitope	VaxiJen score	Antigen-icity	Aller-gen FP Score	Allergen/non-allergen	ToxinPred score	Toxicity
BCPred 12 mer	NS4B	116	AHYAIIGPGLQA	0.8915	antigenic	0.66	allergen	−0.71	Non-toxic
		117	HYAIIGPGLQAK	1.4654	antigenic	0.65	non-allergen	−0.65	Non-toxic
		118	YAIIGPGLQAKA	1.2188	antigenic	0.63	non allergen	−0.67	Non-toxic
		119	AIIGPGLQAKAT	1.2813	antigenic	0.65	non allergen	−0.52	Non-toxic
	NS2b	82	GSMSIKNEEEEQ	1.3688	antigenic	0.66	allergen	−0.92	Non-toxic
	NS1	109	TELKYSWKTWGK	1.0523	antigenic	0.64	non allergen	−1.30	Non-toxic
BCPred 16 mer	NS5	104	TKGGPGHEEPIPMSTY	0.8253	antigenic	0.6	allergen	−1.10	Non-toxic
	NS2b	79	SEDGSMSIKNEEEEQT	1.0438	antigenic	0.61	allergen	−0.93	Non-toxic
	NS1	106	PQPTELKYSWKTWGKA	0.9328	antigenic	0.54	allergen	−1.53	Non-toxic
BCPred 20 mer	NS2b	79	SEDGSMSIKNEEEEQTLTIL	0.9326	antigenic	0.54	allergen	−1.07	Non-toxic
	CAP	105	RPQPTELKYSWKTWGKAKML	0.8398	antigenic	0.6	allergen	−1.10	Non-toxic
AAP method 12 mer	NS4b	150	ITVIDLDPIPYD	1.7819	antigenic	0.66	non allergen	−1.01	Non-toxic
		120	IIGPGLQAKATR	0.9948	antigenic	0.61	non allergen	−0.19	Non-toxic
	ns5	330	KPWDVIPMVTQM	0.9640	antigenic	0.61	non allergen	−0.56	Non-toxic
		591	DIISRRDQRGSG	1.2045	antigenic	0.69	non allergen	−0.99	Non-toxic
	E	389	LSWFKKGSSIGQ	1.1629	antigenic	0.66	allergen	−0.86	Non-toxic
		126	EGKIVQPENLEY	0.8961	antigenic	0.61	allergen	−0.76	Non-toxic
		69	TTASRCPTQGEP	0.9387	antigenic	0.62	allergen	−1.13	Non-toxic
		478	SLVLVGVVTLYL	0.8649	antigenic	0.61	allergen	−1.36	Non-toxic
		156	GKHGKEIKVTPQ	1.5979	antigenic	0.64	allergen	−0.93	Non-toxic

Table 1. (Continued).

Tool	Protein name	Start position	Epitope	VaxiJen score	Antigen-icity	Aller-gen FP Score	Allergen/non-allergen	ToxinPred score	Toxicity
		400	NDWDFVVTTDIS	1.5282	antigenic	0.64	non allergen	−0.97	Non-toxic
		194	HPGAGKTKRYLP	0.8506	antigenic	0.59	non allergen	−0.56	Non-toxic
		354	FKGKTVWFVPSI	0.9641	antigenic	0.64	non allergen	−1.26	Non-toxic
		11	PPVGKAELEDGA	0.8332	antigenic	0.6	allergen	−0.78	Non-toxic
		35	QIGAGVYKEGTF	0.8303	antigenic	0.66	non allergen	−0.63	Non-toxic
		71	TSTWVTYGTCTATGEHRREK	0.8888	antigenic	0.63	allergen	−0.61	Non-toxic
	ancC	35	LGMLQGRGPLKL	0.9718	antigenic	0.61	allergen	−1.05	Non-toxic
	NS1	2	SGCVVSWKNKEL	1.5320	antigenic	0.63	allergen	−0.49	Non-toxic
		249	GPVSQHNYRPGY	1.2320	antigenic	0.64	allergen	−0.80	Non-toxic
		330	WYGMEIRPLKEK	0.8858	antigenic	0.59	non allergen	−0.63	Non-toxic
AAP method 16 mer	ns4b	148	DGITVIDLDPIPYDPK	1.4923	antigenic	0.59	non allergen	−0.86	Non-toxic
	NS5	249	KATYEPDVDLGSGTRN	1.2011	antigenic	0.61	allergen	−0.51	Non-toxic
	E	315	TQHGTIVVRVQYEGDG	0.8382	antigenic	0.62	allergen	−1.15	Non-toxic
		381	GVEPGQLKLSWFKKGS	1.1286	antigenic	0.59	non allergen	−1.73	Non-toxic
		360	EKDSPVNIEAEPPFGD	0.9236	antigenic	0.56	non allergen	−1.31	Non-toxic
		245	AKKQDVVVLGSQEGAM	0.8631	antigenic	0.57	non allergen	−0.93	Non-toxic
		126	EGKIVQPENLEYTIVV	0.9352	antigenic	0.56	non allergen	−0.83	Non-toxic
	NS2A	385	GQLKLSWFKKGSSIGQ	1.0234	antigenic	0.65	non allergen	−1.20	Non-toxic
		363	SPVNIEAEPPFGDSYI	0.8477	antigenic	0.57	non allergen	−1.13	Non-toxic
		67	NTTTASRCPTQGEPSL	0.8324	antigenic	0.63	non allergen	−1.07	Non-toxic
		315	TQHGTIVVRVQYEGDG	0.8382	antigenic	0.62	allergen	−1.15	Non-toxic
		245	AKKQDVVVLGSQEGAM	0.8631	antigenic	0.57	non allergen	−0.93	Non-toxic
		126	EGKIVQPENLEYTIVV	0.9352	antigenic	0.56	non allergen	−0.83	Non-toxic
	NS3	130	FSPGTSGSPIVDKKGK	1.0078	antigenic	0.6	non allergen	−0.69	Non-toxic
		99	ALEPGKNPRAVQTKPG	0.8707	antigenic	0.54	non allergen	−1.30	Non-toxic
		395	IKTRTNDWDFVVTTDI	1.5577	antigenic	0.64	non allergen	−0.63	Non-toxic
		61	KGKRIEPSWADVRKDL	1.1855	antigenic	0.57	non allergen	−1.10	Non-toxic
		240	IRYQTPAIRAEHTGRE	1.0243	antigenic	0.57	non allergen	0.32	Toxic
	PREM	80	EDGSMSIKNEEEEQTL	1.0234	antigenic	0.56	allergen	−1.01	Non-toxic
	ancC	4	QRKKARNTPFNMLKRE	0.9848	antigenic	0.59	non allergen	−1.30	Non-toxic
	NS1	106	PQPTELKYSWKTWGKA	0.9328	antigenic	0.54	allergen	−1.53	Non-toxic
		1	DSGCVVSWKNKELKCG	1.6065	antigenic	0.55	allergen	−0.65	Non-toxic
		329	CWYGMEIRPLKEKEEN	1.1714	antigenic	0.52	allergen	−0.47	Non-toxic
		164	TTNIWLKLKERQDVFC	1.2888	antigenic	0.6	allergen	−0.95	Non-toxic
AAP method 20 mer	NS5	451	VYNMMGKREKKMGEFGKAKG	0.9994	antigenic	0.63	non allergen	−0.40	Non-toxic
	E	382	VEPGQLKLSWFKKGSSIGQM	1.0872	antigenic	0.67	non allergen	−1.26	Non-toxic
		353	TVNPIVTEKDSPVNIEAEPP	0.9435	antigenic	0.64	allergen	−1.15	Non-toxic
		311	EIAETQHGTIVVRVQYEGDG	0.8451	antigenic	0.65	non allergen	−1.11	Non-toxic
		61	IEAKLTNTTTASRCPTQGEP	1.0315	antigenic	0.65	non allergen	−0.94	Non-toxic
		187	PRTGLDFNEMVLLQMENKAW	0.9880	antigenic	0.65	non allergen	−1.20	Non-toxic
	NS3	448	PVTHSSAAQRRGRIGRNPKN	0.9498	antigenic	0.65	non allergen	−0.60	Non-toxic
		397	TRTNDWDFVVTTDISEMGAN	1.1820	antigenic	0.68	allergen	−0.57	Non-toxic
	preM	71	TSTWVTYGTCTATGEHRREK	0.8888	antigenic	0.63	allergen	−0.61	Non-toxic
	CAP	100	GKRSLRPQPTELKYSWKTWG	1.3722	antigenic	0.6	allergen	−1.31	Non-toxic
		1	DSGCVVSWKNKELKCGSGIF	1.0805	antigenic	0.61	allergen	−0.88	Non-toxic
		325	GEDGCWYGMEIRPLKEKEEN	0.8346	antigenic	0.57	allergen	−0.22	Non-toxic

Table 2. Predictions of consensus epitope.

Eptide length	Protein	Start position	Epitope	Tool
12 mer	NS2b	82	GSMSIKNEEEEQ	BCPred
20 mer	NS2b	79	SEDGSMSIKNEEEEQT	BCPred
16 mer	PREM	80	EDGSMSIKNEEEEQTL	BCPred
16 mer	PREM	80	EDGSMSIKNEEEEQTL	AAP method
12 mer	NS1	109	TELKYSWKTWGK	BCPred
16 mer	NS1	106	PQPTELKYSWKTWGKA	BCPred
16 mer	NS1	106	PQPTELKYSWKTWGKA	AAP method
12 mer	NS1	2	SGCVVSWKNKEL	AAP method
16 mer	NS1	1	DSGCVVSWKNKELKCG	AAP method
16 mer	NS1	329	CWYGMEIRPLKEKEEN	AAP method
20 mer	CAP	105	RPQPTELKYSWKTWGKAKML	BCPred
20 mer	CAP	1	DSGCVVSWKNKELKCGSGIF	AAP method
20 mer	CAP	325	GEDGCWYGMEIRPLKEKEEN	AAP method
12 mer	E	389	LSWFKKGSSIGQ	AAP method
12 mer	E	400	NDWDFVVTTDIS	AAP method
12 mer	E	126	EGKIVQPENLEY	AAP method
16 mer	E	126	EGKIVQPENLEYTIVV	AAP method
16 mer	E	315	TQHGTIVVRVQYEGDG	AAP method
16 mer	E	245	AKKQDVVVLGSQEGAM	AAP method
20 mer	E	382	VEPGQLKLSWFKKGSSIGQM	AAP method
16 mer	NS2A	385	GQLKLSWFKKGSSIGQ	AAP method
16 mer	NS2A	245	AKKQDVVVLGSQEGAM	AAP method
20 mer	NS3	397	TRTNDWDFVVTTDISEMGAN	AAP method
12 mer	ancC	330	WYGMEIRPLKEK	AAP method
20 mer	NS5	311	EIAETQHGTIVVRVQYEGDG	AAP method
12 mer	NS4b	150	ITVIDLDPIPYD	AAP method
16mer	NS4b	148	DGITVIDLDPIPYDPK	AAP method

Table 3. Predictions of conservancy analysis.

Sl.	Protein	Epitope sequence	Epitope length	% of protein sequence matches	Maximum identity	Minimum identity
1.	E	VEPGQLKLSWFKKGSSIGQM	20	**100.00% (88/88)**	100.00%	100.00%
2.	E	EGKIVQPENLEYTIVV	16	**53.41% (47/88)**	93.75%	100.00%
3.	E	NDWDFVVTTDIS	12	**98.86% (87/88)**	83.33%	100.00%
4.	E	AKKQDVVVLGSQEGAM	16	**98.86% (87/88)**	93.75%	100.00%
5.	NS5	EIAETQHGTIVVRVQYEGDG	20	**75.27% (70/93)**	95.00%	100.00%
6.	NS1	TELKYSWKTWGK	12	**100.00% (97/97)**	100.00%	100.00%
7.	ancC	WYGMEIRPLKEK	12	**1.02% (1/98)**	25.00%	100.00%
8.	NS4b	DGITVIDLDPIPYDPK	16	**96.51% (83/86)**	87.50%	100.00%

Toxicity prediction of B cell

Toxicity analysis was conducted on the ToxinPred web server. Anything expected by the ToxinPred web server as toxic has been discarded. The negative value of the peptide showed that it was non toxic. On the other hand, positive value of the peptide showed that it was toxic. To analyze toxicity of the 66 epitopes, it was expected that 65 were nontoxic (Table 1). Just 1 of the toxic peptides and the majority of the 65 non-toxic epitopes were omitted for further study.

Prediction of the allergenicity

The Allergen FP server was used to forecast the final vaccine construct's non-allergic actions. Forecasting is based on SVM method. 31 allergenic epitopes and 35 non-allergic activity

prediction epitopes were seen out of 66 epitopes by Allergen FPP, using the amino acid sequence for prediction. The BC pred server reveals 7 allergenic and 4 non allergic predictions, while the AApred server shows 24 allergenic and 31 non allergic predictions (Table 1). For further research, only non-allergenic epitopes were used.

Consensus epitopes prediction

In this analysis, a total of 27 consensus epitopes were predicted as shown in Table 2. The AAA method predicted 24 epitopes, while the BCpred method predicted only three. The majority of the consensus 12 epitopes were found to be 16 mer long. The numbers 8 and 7 were 12 and 20 mer, respectively. The E protein has four overlap epitopes, like VEPGQLKLSWFKKGSSIGQM, EGKIVQPENLEYTIV, NDWDFVVTTDIS, and AKKQDVVVLGSQEGAM. Epitopes EIAETQHGTIVVRVQYEGDG, TELKYSWKTWGK, WYGME IRPLKEK, and DGITVIDLDPIPYDPK from NS5, NS1, ancC, and NS4b proteins were also found overlapped.

Conservancy analysis

Conservancy at 70% sequence identity threshold across consensus sequences, 2 out of 8 epitopes showed 100% conservancy across all DENV2 serotypes consensus sequences (Table 3). We found in this study that the epitope VEPGQLKLSWFKKGSSIGQM length 20 mer and TELKYSWKTWGK length 12 mer showed 100% preservation, while the epitope NDWDFVVTTDIS and AKKQDVVVLGSQEGAM length 12 mer and 16 mer showed 98.86% preservation. Other epitope length 16 mer DGITVIDLDPIPYDPK and length 20 mer EIAETQHGTIVVRVQYEGDG display 96.51% and 75.27% Conservancy. Remaining EGKIVQPENLEYTIVV length 16 mer and WYGMEIRPLKEK length 12 mer epitope reveals low Conservancy 53.41% and 1.02% discarded from the vaccine selection process, respectively. All the predicted B cell epitopes were then supplied with the IEDB conservancy analysis interface with a sequence identity threshold set at 70%. Both the DENV2 serotype consensus sequences envelope protein and NS5, NS1, ancC and NS4b were selected as homologous protein sets, respectively.

DISCUSSION

Every year, a large portion of the population is infected with one of the dengue virus serotypes, and a substantial number of these people experience serious DSS/DHF (Amorim *et al*., 2016). This illustrates the challenging task of developing a safe and efficient dengue virus vaccine. In contrast to conventional vaccine production, bioinformatics analysis can predict potent epitopes, making vaccine design simple and fast (Rappuoli *et al*., 2016). Within protection of viral infections, humoral immunity plays an essential function, alongside cell-mediated immunity. Recognition of B-cell epitopes is therefore essential in the understanding of viral pathogenesis and in the production of vaccines (Barlow *et al*., 1986). Antigenicity, allergenicity, and toxicity of predicted epitopes were evaluated by the VaxiJen v2.0 tool, Allergen FP server and ToxinPred web-server, respectively. The VaxiJen tool was used to analyze the defensive antigens based on the overall antigenicity score to further improve the prediction (Mehla and Ramana, 2016). Toxic peptide prediction is an important step in the production of a potent epitope-based vaccine. Using the Toxinpred tool, the toxic epitopes were omitted from further analysis. For consensus epitopes, the predicted B cell epitopes in each were also manually compared with each other. Epitope-based vaccines are a safe option for vaccine production since they can stimulate a particular immune response without causing any side effects (Oany *et al*., 2015).

CONCLUSION

VEPGQLKLSWFKKGSSIGQM, TELKYSWKTWGK, NDWDFVVTTDIS, AKKQDVVVLGSQEGAM, EIAETQHG TIVVRVQYEGDG, and DGITVIDLDPIPYDPK epitopes were selected for designing a vaccine against dengue virus. These epitopes had good antigenicity, allergicity, and conservancy. After experimental study, these epitopes may form the basis for the production of a Dengue vaccine.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

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