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In silico prediction of b-cell epitopes of dengue virus – A reverse vaccinology approach

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ABSTRACT

Dengue virus, a mosquito-borne flavivirus, causes dengue fever in humans. There are four dengue serotypes and infection with more than one serotype resulting in severe dengue hemorrhagic fever/dengue shock syndrome. So far, only one vaccine is available for dengue, but its efficacy against all serotypes across various ethnics is not confirmed. A vaccine that can neutralize all four dengue serotypes could be more effective in combating the virus. Prediction of B-cell epitopes using *in silico* tools, and their subsequent identification will enhance our understanding of the disease pathogenesis and in the development of better vaccines. In this work, three different prediction methods, viz., ABCpred, BCpred, and AAP, were employed for the analysis of all four DV proteomes, resulting in the prediction of 10083 B-cell epitopes, out of which 251 were found to be consensus epitopes occurring in more than one DV serotype. The 251 consensus epitopes were further analyzed for toxicity, antigenicity, and overlapping epitope prediction. Among them, 151 epitopes were predicted as antigenic. Six of them were found to be overlapping, i.e., predicted by more than two prediction methods. Analysis using IEDB database indicates that 92 out of 151 predicetd peptides are novel, hitherto unreported peptides.

INTRODUCTION

The dengue virus (DV) is a member of the *flaviviridae* family which is transmitted by two mosquitoes, namely, *Aedes aegypti* and *Aedes albopictus*. Nowadays, DV has been endemic in more than 100 countries, including the Americas, Africa, the Western Pacific, Southeast Asia, and Eastern Mediterranean (Yauch *et al*., 2009). It was estimated that 390 million dengue infection occurs every year and 500,000 dengue-related cases have been hospitalized. Therefore, at present, dengue is often considered as a major arboviral disease in the world (Priyamvada *et al*., 2016). There are four serotypes (DV-1, 2, 3, and 4), all of which cause classical dengue fever to severe forms of dengue hemorrhagic fever and dengue shock syndrome (DHF/DSS) (McBurney *et al*., 2016). Most of the studies indicate that DV infection can provoke not only neutralizing antibody response but also non-neutralizing antibodies (Duan *et al*., 2015). The DV has unique antibody epitopes, which are more specific to each serotypes. Individuals who have recovered from primary infection have developed long-term protective immune response against particular homologues serotypes (Wahala *et al*., 2010). Such antibodies are capable of preventing the cell entry into the host cell, which is extensively demonstrated *in vitro* either by prevention of conformational change in the protein or blockade of viral binding to host cells (Amorim *et al*., 2016). Non-neutralizing antibodies produced during primary infection can weakly cross-react with the heterologous serotypes and enhance the viral infectivity rate in $Fc\gamma R^{+}$ cells. These phenomena are known as antibody-dependent enhancements, which lead to DHF/DSS (Dejnirattisai *et al*., 2010). Therefore, identification of B-cell epitopes for DV is important as it can contribute to the understanding of immunological responses in DV infection, as well as in providing information for vaccine development.

B-cell epitopes are peptides composed of hydrophilic amino acids present on the protein antigen or other biomolecules which are recognized by soluble or membrane-bound immunoglobulin molecules. These epitopes are focused on not only for pathogenesis and immunological research but also as a main

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target for vaccine and diagnostic reagent development (Jiang *et al*., 2010). At the outset, B-cell epitopes can be classified into continuous and discontinuous. Continuous epitopes are linear epitopes formed by 3–8 amino acid residues representing continuously on the primary structure of the parental protein. Discontinuous epitopes otherwise known as conformational epitopes consist of more than 10 amino acid residues and occur in a discrete manner, but assemble to exhibit an antigenic form in the tertiary structure of the parental protein (Laver *et al*., 1990). Prediction of linear B-cell epitopes with high accuracy is of paramount importantance for epitope-based immunotherapy**.** Several bioinformatics algorithms and servers are available for the predicition of B-cell epitopes. Most of the B-cell epitope prediction algorithms are focused on linear epitopes because it is believed that linear epitopes are capable of eliciting an antibody response that can cross-react with the parental antigen (Saha and Raghava, 2007a; 2007b). Since 1981, many B-cell epitope prediction algorithms have been developed based on various amino acid properties (Hopp and Woods,1981; Hopp and Woods, 1983; Ponomarenko and Van Regenmortel, 2009) such as hydrophilicity (Parker *et al*., 1986), hydrophobicity (Eisenberg *et al*., 1984), antigenicity (Welling *et al*., 1985), solvent accessibility (Emini *et al*., 1985), secondary structure (Chou and Fasman, 1974), flexibility (Karplus and Schulz, 1985), and many others. Few more algorithms based on machine learning techniques like BepiPred, APCpred, ABCpred, LBEEP, and LBtope have been developed recently for the prediction of linear B-cell epitopes (Dhanda *et al*., 2017). Currently, many of the B-cell prediction tools are freely available online. Most of the previous studies on dengue B-cell epitope prediction have employed a single DV protein and algorithm. In the current study, multiple prediction tools, such as ABCpred and BCPREDS, were applied to analyze all the structural and non-structural proteins of DV proteome for the prediction of B-cell epitopes covering all four DV serotypes.

METHODS

Collection of source data

All structural (ACP, PrM, and EPE) and non-structural proteins (NS1, NS2a, NS2b. NS3, NS4a, NS4b, and NS5) of DV were retrieved from GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) and their accession numbers are provided in Table 1. Proteomes of all 4 DV serotypes were analyzed for prediction of B-cell epitopes.

Prediction of linear B-cell epitopes

Potential 12, 14, 16, 18, and 20-mer B-cell epitopes from all the proteins of four dengue serotypes were predicted using two B-cell epitope prediction algorithms, ABCpred (http://www.imtech.res.in/raghava/abcpred/) and BCPREDS (http://ailab.ist.psu.edu/bcpred/predict.html). Complete sequences of each of these proteins were submitted individually to these two servers and the results were recorded. The fixed length patterns are common in both the B-cell epitope prediction servers. Therefore, the fixed length pattern was chosen for this study. BCPREDS includes two methods for fixed length (BCPred and AAP algorithms) and one method for flexible length (FBCPred algorithm). In this study, BCPred and AAP methods were selected for B-cell epitope prediction. The default parameter provided in the servers for determination of B-cell epitope prediction was used.

Consensus epitope prediction

Prediction of common epitopes between or among existing serotypes could be used for the preparation of multivalent vaccine against DV. The results of predicted epitopes (12, 14, 16, 18, and 20-mer) from all four dengue serotypes by each tool were compared with each other and the common peptides found to occur in more than one serotypes were considered as consensus epitopes. The primary reason to use consensus epitope approach was to find putative candidates with higher probability to confer immune response against several serotypes of DV.

Toxicity prediction

The predicted putative candidates must not provoke any toxic effects on humans while administration. Hence, the toxic nature of the predicted epiotpes was evaluated using a web-based Toxinpred server (http://www.imtech.res.in/raghava/toxinpred/design.php). The consensus epitopes predicted by each tool were further filtered based on the results of toxicity prediction. The default parameters provided in the server were selected for this analysis.

Table 1. Dengue viral proteins and their accession numbers.

S. No.	Protein	Accession no.			
		Dengue 1	Dengue 3	Dengue 3	Dengue 4
1	ACP (Anchored capsid protein)	NP_722457	NP_739581.2	YP_001531165.2	NP_740314.1
2	PrM (Premembrane protein)	NP_733807	NP_739582.2	YP_001531166.1	NP_740315.1
3	EPE (Envelope protein E)	NP_722460	NP_739583.2	YP_001531168.2	NP_740317.1
4	NS1 (Nonstructural protein 1)	NP_722461	NP_739584.2	YP_001531169.2	NP_740318.1
5	NS2a (Nonstructural protein 2a)	NP_733808	NP_739585.2	YP_001531170.2	NP_740319.1
6	NS2b (Nonstructural protein 2b)	NP_733809	NP_739586.2	YP_001531171.3	NP_740320.1
7	NS3 (Nonstructural protein 3)	NP_722463	NP_739587.2	YP_001531172.2	NP_740321.1
8	NS4a (Nonstructural protein 4a)	NP_733810	NP_739588.2	YP_001531173.2	NP_740322.1
9	NS4b (Nonstructural protein 4b)	NP_733811	NP_739589.2	YP_001531175.2	NP_740324.1
10	NS5 (Nonstructural protein 5)	NP_722465	NP_739590.2	YP_001531176.2	NP_740325.1

Antigenicity prediction

The predicted B-cell epitopes should be potentially antigenic so that optimal immune response can be elcited by lymphocytes upon exposure to the parental antigen. Therefore, VaxiJen v2.0 (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) server was used to predict the antigenic nature of the predicted epitopes. Default parameters were selected for the determination of antigenic peptides.

Epitope cluster analysis

The consensus B-cell epitopes that were predicted in DV serotypes (1–4) were subjected to cluster analysis. All 12, 14, 16, 18, and 20-mer epitopes were grouped into clusters based on sequence identity using a cluster analysis tool available in IEDB-AR (http://tools.immuneepitope.org/tools/cluster/iedb_input). The density of the cluster was calculated based on the number of predicted consensus epitopes present within a cluster with the threshold sequence similarity of 80% (Yao *et al*., 2013).

Overlapping epitope prediction

The consensus epitopes predicted by each server were compiled into one set and compared with the other server sets which originated from the same protein and apparently common peptides predicted by more than one tool were considered as the most probable multivalent B-cell epitopes.

Accessibility and antigenic propensity analysis

It was observed that antigenic and accessibility regions of antigens interact with the binding site of the antibody. All the consensus epitopes predicted in the present study were further analyzed using the BcePred tool for anlyzing accessibility and antigenicity regions. The default threshold values of 2 (accessibility) and 1.8 (antigenic propensity) were selected.

Conformational B-cell epitope prediction

In order to improve the accuracy of B-cell epitope mapping, the CBTOPE tool was used for mapping of antibody intracting residues of DV antigen. CBTOPE predicts the conformational B-cell epitopes in a given antigen based on its primary amino acid sequence, whereas other prediction tools require the structure of the antigen to predict the conformational epitopes. The structural and non-structural proteins of DV were used to predict the possible conformational epitopes that would be present in the DV proteome. Furthermore, the conformational epitope regions were manually compared with consensus epitopes for the prediction of antibody binding region of predicted linear B-cell epitopes. The default threshold value of −0.3 was selected for this analysis.

Experimental validation of B-cell epitopes

The IEDB database provides information about experimentally validated data of B- and T-cell epitopes for human and non-human primates and other animal species. The consensus B-cell epitopes predicted in the present study were further searched in Immune Epitope Database (http://www.immuneepitope.org) for the identification of reported human B-cell epitopes. The IEDB BLAST search was also carried out against exact and partially matched (90% sequence similarity) with an identified sequence of B-cell epitopes.

RESULTS

Prediction of B-cell epitopes

A total of 10,083 B-cell epitopes were predicted from the proteome of all four DV serotypes (Fig. 1). Among them, the highest number (6,723) of B-cell epitopes was predicted by ABCpred server, followed by 1,765 and 1,595 epitopes predicted

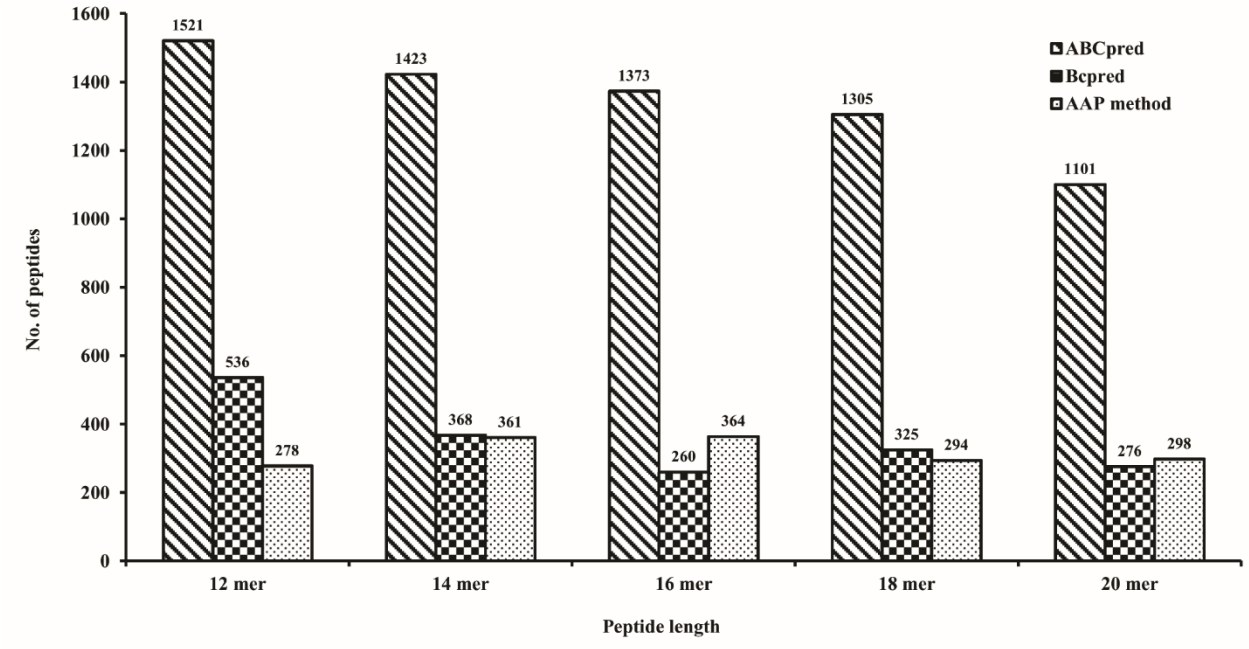

Figure 1. Epitope prediction by ABCpred, BCpred, and AAP methods. The maximum numbers of epitopes were identified in ABCpred analysis.

by BCpred and AAP methods of BCPREDS server, respectively. Out of 10,083 epitopes analyzed by fixed length pattern (12–20 mer), 2,335 (23.16%) epitopes were of 12-mer in length, followed by 2,152 (21.34%), 1,997 (19.81 %), 1,924 (19.08 %), and 1,675 (16.61 %) of 14, 16, 18, and 20-mer length, respectively.

Consensus epitope prediction

A total of 251 consensus epitopes were predicted in this study. The highest number of consensus epitopes (169) was predicted by ABCpred (Supplememtary Table 1), whereas 48 and 34 epitopes were predicted by BCpred (Supplememtary Table 2) and AAP methods (Supplememtary Table 3), respectively. Most of the consensus epitopes (93) were found to be of 12-mer in length, which is 37.05% of total consensus epitopes. This is followed by 46 (18.33%), 73 (29.08%), 21 (8.37%) and 18 (7.17%), which were of 14, 16, 18, and 20-mer, respectively (Fig. 2). Out of 251, 218 epitopes were found to be present in any two of the DV serotypes, whereas 30 epitopes were found to occurr in any three DV serotypes. Interestingly, three epitopes (NS5-DLGCGRGGWSYY, EPE-DRGWGNGCGLFG, and NS4b-IIGPGLQAKATREAQK) were found to be present in all four DV serotypes (Fig. 3).

Toxicity prediction of B-cell epitopes

All the consensus epitopes were further analyzed by Toxinpred tool for prediction of toxic peptides. Out of the 251 consensus epitopes, 244 were predicted to be non-toxic (Supplementary Material 4). The 7 toxic peptides were excluded and the rest of the 244 non-toxic epitopes were used for further analysis.

Antigenicity prediction of B-cell epitopes

The antigentic nature of the shortlisted consensus epitopes were further analyzed using the VaxiJen (v2.0) tool. A total of 151 antigenic epitopes were predicted out of the 244 consensus epitopes analyzed (Supplementary Material 5). Among this, the highest numbers of antigenic consensus epitopes (99) were predicted by ABCpred, followed by BCpred (30) and AAP method (22). The consensus epitopes predicted by each method exhibited varying degrees of antigenicity: 0.2491 to 2.046 for ABCpred, 0.4483 to 1.6726 for BCpred, and 0.4172 to 1.1243 for AAP methods. In ABCpred analysis, 29.29% (29 out of 99) of the epitopes showed an antigenic score of more than 1. In BCpred and AAP analysis, 36.67% (11 out of 30) and 27.27% (6 out of 22) of epitopes, respectively, exhibited an antigenic score of more than 1. The NS3 epitope, AIALDFKPGTSGSP, predicted by ABCpred exhibited a highest antigenic score of 2.046, out of 244 epitopes analyzed by all three prediction methods.

Overlapping epitope prediction

Only 6 epitopes out of 244 were found be overlapping among the four serotypes of DV (Table 2). Out of the six, five epitopes were predicted by two of the three prediction methods. Only one 16-mer epitope, GKREKKLGEFGKAKGS, part of NS5 protein, was predicted by all three prediction methods. The overlapping epitopes predicted in this study are present in more than one DV serotype, except DV-4.

Accessibility and antigenic propensity analysis

Out of the 244 consensus epitopes analyzed, 127 epitopes were found to contain accessibility regions (Supplementary

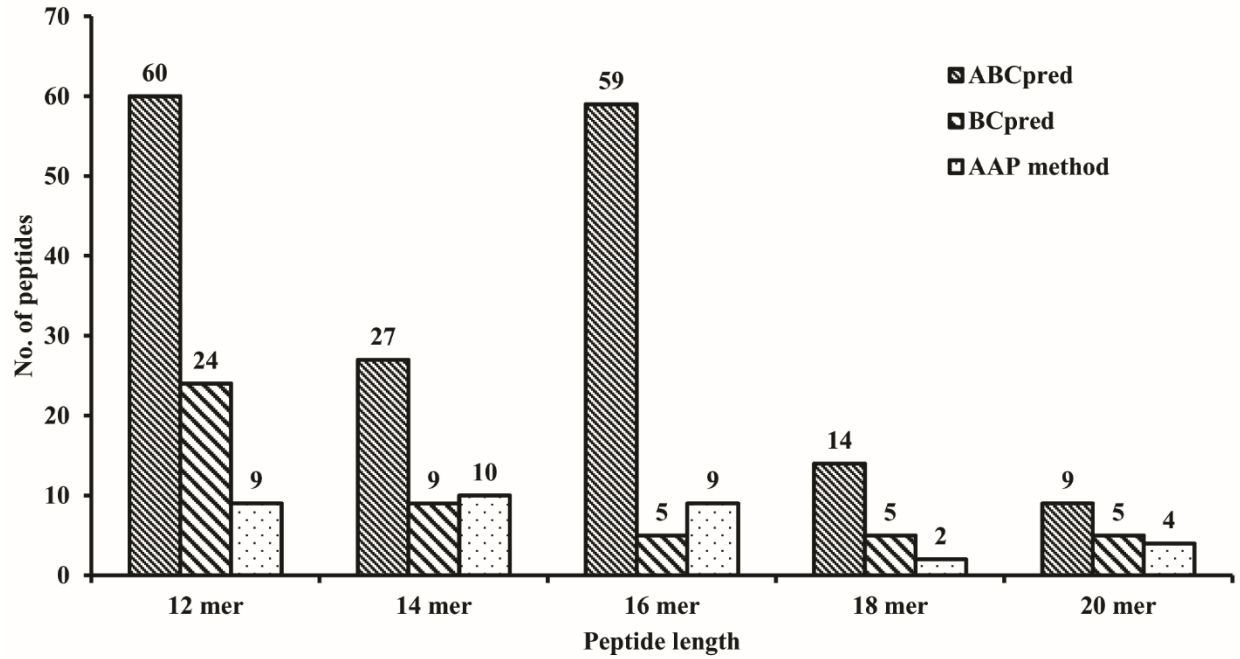

Figure 2. Identification of consensus epitopes among predicted epitopes. The highest numbers of consensus peptides predicted were of 12 mer in length.

Material 6a–c). The highest numbers (82) of accessibility regions were predicted in ABCpred analysis, followed by BCpred (25) and AAP method (20), whereas only 51 antigenic epitopes were predicted out of 244 consensus epitopes analyzed. Among this, the highest numbers of antigenic epitopes (37) were predicted in ABCpred analysis; this is followed by 9 and 5 epitopes predicted using the BCpred and AAP method, respectively. Intrestingly, 19 epitopes contain both surface accessibility and antigenic properties as predicted by all three prediction methods (Table 3).

Conformational epitope prediction

A total of 150 conformational epitopes were identified out of 244 consensus epitopes predicted in this study. The highest numbers of conformational epitopes (96 out of 244) were predicted in ABCpred analysis, followed by 29 and 25 conformational epitopes identified using BCpred and AAP methods, respectively. Most of the conformational epitopes (54) were found to occur as 12-mer in length which is 36% of the total epitopes; this is followed by 32.67% (49), 12,67% (19), 9.33% (14), and 9.33% (14) of 16-mer, 14-mer, 18-mer, and 20-mer, respectively.

Epitope cluster analysis

All the consensus epitopes predicted by ABCpred, BCpred, and AAP methods were grouped into 81 clusters (Supplementary Material 7). The predicted epitopes with 80% sequence similarity formed a cluster. The maximum numbers (12) of consensus epitopes were found to occur in cluster 17; cluster 7 contained 9 consensus epitopes that showed 80% sequence similarity which was identified by three different epitope prediction methods. This is followed by cluster 19, which contained 7 epitopes in their group; clusters 10 and 24 consisted of 6 epitopes each in their cluster groups.

Experimental validation of B-cell epitopes

The consensus epitopes predicted by all three prediction methods were further analyzed using the IEDB tool for exact and partial matching with experimentally proven B-cell epitopes. Interestingly, although none of the consensus epitopes were shown as experimentally proven in exact match search, 59 of the 244 consensus epitopes were shown as experimentally proven with 90% similarity in the IEDB-BLAST search (Supplementary

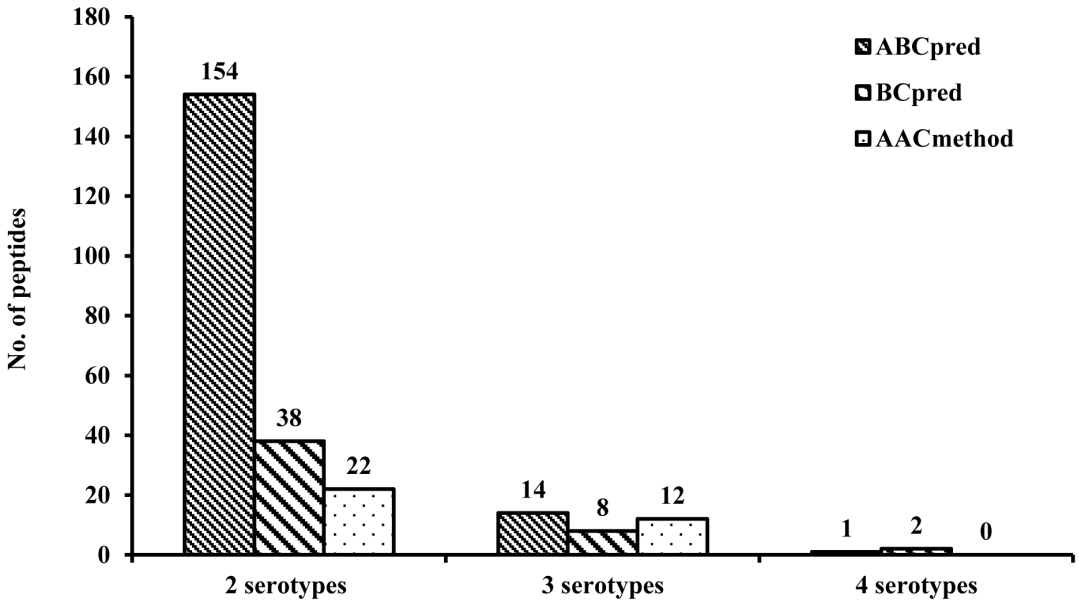

Figure 3. Consensus epitopes predicted among the four DV serotypes.

Table 2. Overlapping epitopes predicted by more than one prediction method.

Peptide length	Protein	DV Serotype	Start position	Peptide	Tools[a]
12 mer	EPE	1/3	100/100	GWGNGCGLFGKG	A/C
	NS1	1/3	21/21	VTNEVHTWTEQY	A/B
	NS5	1/3	89/89	YYCAGLKKVTEV	A/C
14 mer	NS3	2/3	284/285	DEAHFTDPASIAAR	A/C
16 mer	NS5	1/2/3	455/456/455	GKREKKLGEFGKAKGS	A/B/C
	NS5	2/3	333/332	DVVPMVTQMAMTDTTP	A/C

[a]Tools used: A-ABCpred; B-BCpred; C-AAC method (Bcpred).

Table 3. Overlapping epitopes containing both accessibility and antigenicity regions.

S. No	Length	Protien	Serotype	Position	Epitope	BcePred		Experimetal Evidence (IEDB search)
						Accessibility	Antigenicity	PubMed ID
					BCpred			
1	12 mer	EPE	1,3	243/241	AHAKKQEVVVLG	**AHAKKQEVV**VLG	AHA**KKQEVVVLG**	19826631, 7505071
2		NS 3	2,3	271/272	SPVRVPNYNLII	SP**VRVPNYNLII**	**SPVRVPNYNLII**	NR
3	20 mer	NS 3	2,3	260/261	MCHATFTMRLLSPVRVPNYN	MCHATFTMRLLSP**VRVPNYN**	MCHATFT**MRLLSPVRVPNYN**	NR
					AAP method			
4	12 mer	NS 3	2,3	269/270	LLSPVRVPNYNL	LLSP**VRVPNYNL**	**LLSPVRVPNY**NL	NR
5	16 mer	EPE	1,3	243/241	AHAKKQEVVVLGSQEG	**AHAKKQEVV**VLGSQEG	AHA**KKQEVVVLGSQE**G	19826631, 7505071, 2472749
					ABCpred			
6	12 mer	NS3	3,4	380/379	GKKVIQLSRKTF	GKKV**IQLSRKTF**	G**KKVIQLSRK**TF	
7		NS4b	2,3	163/63	KFEKQLGQVMLL	**KFEKQLGQ**VMLL	KFEK**QLGQVMLL**	NR
8		NS5	1,2	143/143	LLCDIGESSPNP	LLCDI**GESSPNP**	**LLCDIGE**SSPNP	NR
9	14mer	NS3	2,3	267/268	MRLLSPVRVPNYNL	MRLLSP**VRVPNYNL**	**MRLLSPVRVPNY**NL	NR
10		NS5	1,3	201/201	GGMLVRNPLSRNST	GGML**VRNPLSRNST**	GGM**LVRNPLS**RNST	NR
11	16 mer	NS3	1,4	538/537	MRRGDLPVWLSYKVAS	**MRRGDLP**VWLSYKVAS	MRRG**DLPVWLSYKV**AS	NR
12			2,3	375/376	LRKNGKKVIQLSRKTF	**LRKNGKKVIQLSRKTF**	LRKNG**KKVIQLSRK**TF	NR
13			2,4	138/135	AAGIMKNPTVDGITVI	AAGI**MKNPTVDG**ITVI	AAGIMKNPT**VDGITVI**	24503088
14			3,4	163/160	KFEKQLGQVMLLVLCA	**KFEKQLGQ**VMLLVLCA	KFEK**QLGQVMLLVLC**A	NR
15		NS5	1,2	136/136	PPEKCDTLLCDIGESS	**PPEKCDT**LLCDIGESS	PPE**KCDTLLCDIGE**SS	NR
16			1,4	155/155	TIEEGRTLRVLKMVEP	**TIEEGRTLR**VLKMVEP	TIEEGRT**LRVLKMVEP**	NR
17			2,3	840/840	GKREDQWCGSLIGLTS	**GKREDQWC**GSLIGLTS	GKRED**QWCGSLIGL**TS	NR
18	20 mer	NS1	1,3	13/13	LKCGSGIFVTNEVHTWTEQY	LKCGSGIFVTNEV**HTWTEQY**	LK**CGSGIFV**TNEVHTWTEQY	28381638, 22546090, 21519123, 20079511, 18160621, 17329445, 7944953
19		NS5	1,3	198/198	RKHGGMLVRNPLSRNSTHEM	RKHGGML**VRNPLSRNSTHEM**	RKHGGM**LVRNPLS**RNSTHEM	NR

NR = Not reported; the accecibility/antigenic region are highlighted in bold.

Material 8). A total of 164 consensus epitopes were identified in ABCpred, and among this 26.83% (44/164) of the epitopes were experimentally proven as B-cell epitopes, which belong to various DV serotypes; 17.02% (8/47) and 21.21% (7/33) were identified by BCpred and AAP method, respectively, and they were already proven experimentally.

DISCUSSION

A large portion of the population are infected with any one of DV serotypes every year and a significant amount of them develop severe forms of DSS/DHF (Amorim *et al*., 2016). This underscores the development of a safe and effective vaccine against DV which is a challenging task. In December 2015, the first dengue vaccine, Dengvaxia® (CYD-TDV) licensed by Sanofi Pasteur, entered into the market and it has been approved in a few countries, including Mexico, Brazil, and Philippines (Vannice *et al*., 2016). This vaccine could be administrated to people between 9 and 45 years of age (Carvalho *et al*., 2016). In a phase IIb study in Thailand, the vaccine showed lower efficacy in younger children (Schwartz *et al*., 2015). It was suggested that CYD-TDV may increase the risk of hospitalization when administered to children below nine years of age (Carvalho *et al*., 2016).

Humoral immunity also plays a significant role in the prevention of viral infections along with cell-mediated immunity and hence identification of B-cell epitopes is important in the understanding of viral pathogenesis and in vaccine development (Barlow *et al*., 1986). The ability of epitope-based vaccines in stimulating a specific immune response without any side effect make them as a good choice for vaccine development (Oany *et al*., 2013).

Studies conducted in recent years, on B-cell epitope prediction, involved the analysis of a single serotype or protein of DV mainly focusing NS1 and E protein (Gromowski *et al*., 2008; Jiang *et al*., 2010; Matsui *et al*., 2009). In these investigations, only a limited number of epitopes were reported. But the ideal dengue vaccine must be tetravalent and should provoke immune response against all four dengue serotype and the vaccines might not increase the risk of DHF/DSS (Guy *et al*., 2016). In the present

study, a total of 10,083 linear B-cell epitopes were predicted from all 4 DV proteomes using ABCpred, BCpred, and AAP methods. The B-cell epitopes predicted in each serotype were manually compared with one another for consensus epitopes. A total of 251 consensus epitopes were predicted; among this, 214 and 34 epitopes were present in DV-2 and DV-3 serotypes, respectively. Surprisingly, three epitopes, viz., NS5-DLGCGRGGWSYY (predicted by ABCpred), EPE-DRGWGNGCGLFG, and NS4b-IIGPGLQAKATREAQK (predicted by BCpred) were found to occur in all four DV serotypes. Two of these peptides, NS5-DLGCGRGGWSYY and EPE-DRGWGNGCGLFG, were already identified as conserved in all four DV serotypes (Khan *et al*., 2008). Here, we report a novel epitope, NS4b-IIGPGLQAKATREAQK, which is conserved in all four DV serotypes. The consensus epitopes predicted in this study may be useful for the preparation of a multivalent vaccine.

Prediction of toxic peptides is an important step in developing a potent epitope-based vaccine. Therefore, the 251 consensus epitopes were further analyzed using the Toxinpred tool in which 7 epitopes were predicted as toxic and were excluded from further study. To further strengthen the prediction, the VaxiJen tool server was used to analyze the protective antigens based on the overall antigenicity score (Mehla and Ramana 2016). The non-toxic epitopes were further analyzed using VaxiJen server and the peptides with high antigenicity values were considered to be potent B-cell epitopes. Forty-six potent B-cell epitopes were predicted by all three prediction methods with their VaxiJen score of more than 1. Interestingly, few epitopes from ABCpred [PrM-HRRDKRSVALAP (1.5287), NS5-VMDIISRKDQRG NS3-AIALDFKPGTSGSP (2.046), and NS3-RNLTIMDLHPGSGKTR (1.6313)], BCpred [EPE-CVGVGNRDFVEG (1.6726), NS5-KREKKLGEFGKA (1.6453), and NS5-SLMYFHRRDLRL (1.5446)], and AAP [NS3-IMDLHPGSGKTR (1.6043) and NS5-MDIISRKDQRGS (1.5131)] showed antigenicity scores of more than 1.5.

It is believed that the combination of more than one property provides a better accuracy in epitope prediction (Saha and Raghava, 2004). Therefore, the predicted consensus epitopes were further analyzed for identification of accessibility and antigenic propensity using BcePred tool. Intrestingly, 52.04% (127 out of 244) of consensus epitopes have an accessibility region, whereas only 20.90% (51 out of 244) of the consensus epitopses were found to have an antigenic propensity region. The entire region of the following peptides had accessibility: NS5-TPFGQQRVFKEK and NS3-DEERDIPERSWNSG predicted by BCpred; NS5-VRNPLSRNSTHEMY and GKREKKLGEFGKAKGSRA predicted by AAP method; NS5-MMGKREKKLGEF, TPFGQQRVFKEKVDTR, and NS3-LRKNGKKVIQLSRKTF. Furthermore, 19 epitopes were found to contain both accessibility and antigenicity properties out of 244 epitopes analyzed in this study. Interestingly, all the 19 epitopes were found to be non-toxic in nature. Hence, these epitopes may be useful for the preparation of a DV-specific vaccine preparation.

Conformational epitopes play an important role in peptide-based vaccine development. It is also known that ~90 of B-cell epitopes were confomational epitopes (Ansari and Raghava, 2010). In this study, the antibody interaction region identified in DV proteome was further compared with consensus epitopes for the identification of accurate B-cell epitopes. Majority of the linear epitopes (150 out of 251) were predicted as conformational epitopes. Intrestingly, the entire region of NS5 epitope YYCAGLKKVTEV predicted by AAP method and ABCpred and the EPE epitope DRGWGNGCGLFG predicted by BCpred method was found to be conformational epitopes.

Combination of immune dominant epitopes in a vaccine can elicite a broader immune response to heterologous serotypes (Schussek *et al*., 2014). The cluster analysis indicate that cluster 7 contains 9 epitopes of the DV-NS1 protein: TWTEQYKFQADSPK, CGSGIFVTNEVHTWTE, SGIFVTNEVHTWTEQYKF, LKCGSGIFVTNEVHTWTEQY, and VHTWTEQYKFQA predicted by ABCpred; FVTNEVHTWTEQYK and SGIFVTNEVHTWTEQY predicted by AAP method; VTNEVHTWTEQY and VTNEVHTWTEQYKFQADS predicted by BCpred. These epitopes have common amino acid sequence VHTWTEQYK. Previous studies have reported that VHTWTEQYK epitope could enhance the antibody response against DV in mice and human (Falconar, 2007; 2012). Similarly, cluster 17 contains 12 epitopes of NS2b protein. Of these, a 16-mer epitope IIGPGLQAKATREAQK predicted by BCpred was found to occur in all four DV serotypes. Interestingly, part of this epitope IIGPGLQAKATREA was identified as epitope by all 3 prediction methods. Likewise, cluster 24 contains 6 epitopes of NS5 protein and was found to occur in DV 1, 2, and 3 serotypes. Out of 6 epitopes, 5 epitopes have common amino acid sequence KREKKLGEFGKA which is predicted by all three prediction methods.

The possibility of a predicted epitope to be a conformational epitope is more if the epitope is predicted by more than one tool. Hence, the predicted consensus epitopes were further analyzed by overlapping epitope prediction method. Only 6 overlapping epitopes were predicted out of 244 consensus epitopes analyzed. Incidently, all the six peptides were predicted as epitopes by more than one method. EPE-GWGNGCGLFGKG, and NS1-VTNEVHTWTEQY epitopes were predicted as non-antigenic by VaxiJen tool, whereas the epitopes NS3- DEAHFTDPASIAAR, NS5-YYCAGLKKVTEV, NS5-GKREKKLGEFGKAKGS, and NS5-DVVPMVTQMAMTDTTP exhibited an antigenic score of 0.7001, 0.8325, 1.0836, and 0.9119, respectively.

In addition, all 244 consensus epitopes were searched against IEDB server which provides the information about experimentally validated B-cell epitopes studied in human and non-human primates and in other animal species (Kim *et al*., 2012). None of the epitopes showed as experimentally proved in exact match blast analysis. Whereas, 24.18% (59/244) of epitopes were shown as experimentally proved in 90% similarity blast analysis. The EPE epitope FKNPHAKKQDVVVLGSQEGAMHT and PEVVVLGSQEGAMHT were experimentally proved as potent B-cell epitopes against DV tested in mice (da Silva, 2009) and human (Innis *et al*., 1989). The sequence, SGATWVD, present in the predcited DV EPE epitope (SGATWVDVVLEH) is also reported to be present in Murray valley encephalitis virus epitope, EGASGATWVDLVLEGDSCITI, that was already reported as B-cell epitope in mice (Mathews *et al*., 1991; Roehrig *et al*., 1989). Though 59 of the 151 antigenic epitopes are experimentally

proven as potent B-cell epitopes, the remaining 92 peptides are potential novel epitopes that if confirmed experimentally could pave way for a more potent DV vaccine.

CONCLUSION

A universal vaccine combining many antigenic epitopes that can elicit a broader neutralizing antibody response to all four DV serotypes is needed to combat the virus. To our knowledge, this is the first report on genome-wide mapping of linear B-cell epitopes for all four DV proteomes. Ninty-two potential novel epitopes have been predicted in this study. These epitopes, after experimental analysis, could form a base for the development of a multivalent vaccine against DV.

CONFLICT OF INTEREST

The authors declared that they have no conflict of interests associated with this publication.

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