Abstract

Objective: Human Enterovirus D-68 (EV-D68) is a historically rarely reported virus linked with respiratory disease. However, in the recent years, a large increase in respiratory disease associated with EV-D68 has been reported, with documented outbreaks in North America, Europe and Asia. This study therefore aims to design specific peptide vaccine(s) against the virus by targeting capsid protein VP1 which plays crucial role in host-pathogen interaction.

Material and Methods: Different web-based tools were applied to predict B and T cell epitopes with high accuracy and precision from sequence based analyses of the conserved regions of the capsid protein. Initially MEGA and SWISS-MODEL were used for the sequence and 3D structure analysis respectively. Later, the Immune Epitope Database and Analysis Resource (IEDB-AR), BepiPred and ABCPred servers were used for the identification of T-cell and B-cell epitopes.

Results: “INPADT” peptide was found to be the most potential linear B cell epitope which fulfilled all the criteria of accessibility, hydrophilicity, flexibility and beta turn region for becoming an ideal B cell epitope. On the other hand, “YMSIANANY”, “LVSKRSFEY”, “ENFLSRAAL” and “AMFVPTGAL” were found to be most suitable T cell epitopes interacting with a large number of MHC class I and class II alleles. Among them “LVSKRSFEY” and “ENFLSRAAL” had the highest population coverage. All of these epitopes were found non allergen and with 100% conservancy among different strains of EVD-68 worldwide.

Conclusion: Based on the present study, it could be concluded that these predicted epitopes may be used to design a vaccine against Enterovirus and thus, can be validated in model hosts to verify their efficacy as vaccine.

Key words: Epitope, Enterovirus D68, vaccine, MHC Class I, MHC Class II, T-cell epitopes, B-cell epitopes.

*Corresponding Author: Tahirah Yasmin, Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka-1000, Bangladesh.
1. Introduction

Human enterovirus D68 (EV-D68) is a member of species enterovirus D (EV-D), which belongs to the genus Enterovirus and the family of Picornaviridae. Picornaviruses constitute a large family of small, icosahedral viruses with a single, positive-stranded RNA genome and an external diameter of about 300Å [1] and can cause a wide range of clinical symptoms, ranging from mild febrile illness to fatal meningitis and encephalitis [2]. Although EV-D68 was first isolated from samples obtained in California in 1962 from four children with pneumonia and bronchiolitis [3], it has been rarely reported since then.

However, an upsurge of EV-D68 cases in the past few years worldwide including Japan, the Philippines and the Netherlands [4] as well as several clusters in the USA has implicated EV-D68 as an emerging respiratory pathogen. In August 2014 an outbreak of mild to severe respiratory illnesses occurred among thousands of young children in the United States of which 1116 cases have been confirmed to be caused by EV-D68. This virus has also been associated with occasional neurological infections [5]. Although EV-D68 has emerged as a considerable global public health threat, there is no available vaccine or effective antiviral treatment [1]. Due to the ever rising spread of this viral infection, the development of vaccines or antiviral drugs against this pathogen is crucial and an immediate need.

The capsids of EVs consist of 60 copies of each of four different viral proteins, VP1, VP2, VP3 and VP4. The antigenic diversity among the enteroviruses is caused by variations within capsid proteins VP1 to VP3, but neutralization epitopes are most densely clustered on VP1 [6]. In addition, the hydrophobic pocket of VP1 contains a lipid moiety termed the “pocket factor,” which likely stabilizes the mature virion. As observed in poliovirus and certain picornaviruses, receptor binding at this junction site triggers the uncoating process, which is characterized by the delivery of the viral genome into the host cell compartment for replication and transcription [7].

Based on the consensus that VP1 is an important site for interaction with host cells [8], in this study VP1 capsid protein has been targeted in order to predict informative epitopes which can be helpful for future vaccine designing. The concept of peptide vaccines is based on identification and chemical synthesis of B-cell and T-cell epitopes which are immunodominant and can induce specific immune responses. Well characterized protective epitopes designed from this protein can be a great help for offering consistent, cost effective and quality therapeutics against this pathogen.

Conventional techniques for vaccine development are laborious and time consuming. As a result, computational methods [9] for predicting epitopes have attracted attention of the researchers to reduce the cost and time of vaccine development to fight with the rapidly growing devastating organisms. Current immunoinformatics tools are able to predict human B-cell and T-cell epitopes with high accuracy. These tools are playing a vital role in understanding the molecular basis of immunity and, notably in the development of epitope based-peptide vaccines, immunotherapy against cancer and autoimmune diseases [10]. In comparison to the conventional vaccines, peptide or epitope based vaccines are also easy to develop, chemically stable, more

Specific, and free of any infectious or oncogenic potential hazard [11]. Therefore this present study was undertaken with a view to applying immunoinformatics by using currently available online algorithms in order to identify potentially immunogenic T and B cell epitopes in VP1 capsid protein and design possible candidate peptide vaccines for EV-D68.

2. Materials and Methods

2.1 Retrieval of VP1 protein sequence and identification of conserved regions

The amino acid sequence of VP1 capsid protein of Enterovirus D68 from different strains was retrieved from NCBI database (www.ncbi.nlm.nih.gov/) in FASTA format. To find the conserved region, retrieved sequences were aligned using ClustalW tool that uses Gonnet matrix in MEGA software (v 6.0) along with 1000 bootstrap value and default parameters. For the analysis of the evolutionary divergence in the capsid protein of EVD68, a phylogenetic tree was also constructed using MEGA software.

The minimum length of the conserved regions was set at 15 as binding predictions for MHC class II alleles use to create 15mers. The conserved regions were then used for B and T cell epitope prediction.

2.2 Prediction of putative B cell epitopes, their antigenicity and transmembrane properties

Prediction of potentially immunogenic epitopes in a given protein sequence may significantly reduce wet lab effort needed to discover the epitopes required for the design of vaccines. The aim of the prediction of the B cell epitope was to find the potential antigen that would interact with B lymphocytes and initiate an immune response [12].

For this, antigenicity characteristics of each conserved region were screened using an online antigen prediction server, Vaxijen v2.0 (http://www.ddg-pharmfac.net/vaxijen/). The cut off value for being a probable antigen was set at 0.5. On the other hand, each sequence was also subjected to TMHMM v0.2 server [13] in order to find out whether they contained any transmembrane topology.

In order to predict linear B cell epitopes two different online tools were employed—Bepipred and ABCpred server (http://www.imtech.res.in/raghava/abcpred/). BepiPred predicts the location of linear B-cell epitopes using a combination of a hidden Markov model and a propensity scale method [14]. Default threshold value 0.35 was used for analysis. On the other hand, ABCpred server uses artificial neural network [15] and here cutoff value was set at 0.51.

2.2.1 Prediction of surface accessibility, hydrophilicity, flexibility and beta-turn of the predicted epitopes

A B-cell epitope is characterized by being antigenic, hydrophilic, and accessible in a flexible region of an immunogen [16]. Therefore to find out whether the predicted epitopes possessed these desired characteristics, several tools all hosted by IEDB-AR were utilized. The tools include—Emini surface accessibility prediction [17], Parker hydrophilicity scale [18], Karplus and Schulz flexibility scale [19] and Chou and Fasman beta-turn prediction tool [20] all with default parameters. The results from all these sites were cross-referenced and apparently common findings were taken as the most probable B-cell epitopes.
2.2.2 Structure analysis

The 3D structure of VP1 capsid protein was constructed by homology modeling using the SWISS-MODEL (http://swissmodel.expasy.org) work space. Being a server for automated comparative modeling of three-dimensional (3D) protein structures SWISS-MODEL provides several levels of user interaction where template selection, alignment and model building are done completely automated by the server [21]. Finally, the predicted model was evaluated by using two software tools, PROCHECK [22] and QMEAN [23].

2.3 Prediction of putative T cell epitopes

Firstly, the NetCTL v1.2 server was used for predicting potential cytotoxic T lymphocyte (CTL) epitopes from the conserved peptides which integrates prediction of peptide MHC class I binding, proteasomal C terminal cleavage and TAP transport efficiency [24]. MHC-I binding and proteasomal cleavage were carried out through artificial neural networks and the weight matrix was used to estimate the TAP transport efficiency. The threshold value for epitope identification was set at 0.75 for maintaining sensitivity and specificity of 0.80 and 0.97, respectively during the analysis. The epitope prediction was confined to 12 MHC-I super types.

For cross checking, a similar tool from IEDB analysis resources which combines MHC class I binding, transporter of antigenic peptides (TAP) transport efficiency, and proteosomal cleavage prediction, was employed to predict a total score (http://tools.immuneepitope.org/mhci/) for each conserved peptide’s intrinsic potential of being a T cell epitope [25]. Here, to calculate the half-maximal inhibitory concentration (IC\textsubscript{50}) values Stabilized Matrix Method (SMM) was applied. All the available MHC alleles were selected and the peptide lengths were set at 9.0. The top scoring peptides were selected by setting cut-off values of IC\textsubscript{50} within 100 nM and the overlapping peptides found in both NetCTL and IEDB analysis were subjected to further analysis.

2.3.1 MHC-I and MHC-II restriction analysis

The candidate peptides were analyzed by IEDB MHC class I binding prediction tool (http://tools.immuneepitope.org/mhci/) for all the available class I alleles. The conserved region of each protein was also tested for predicting epitopes that interact with MHC class II molecules by selecting all the alleles in IEDB MHC class II binding prediction tool (http://tools.immuneepitope.org/mhcii/). In both cases, SMM-align method was employed to find out good binders and the cut-off value of IC\textsubscript{50} was set at 100 nm.

2.3.2 Analysis of population coverage

Population coverage for the selected epitopes was assessed by the IEDB population coverage calculation tool [26]. Here the allelic frequency of the interacting HLA alleles was used for the prediction of the population coverage of the corresponding epitopes.

2.4 Allergenicity and disorder analysis of the conserved regions

The web-based Allerdctor server was used to predict the allergenicity of the predicted epitopes for vaccine development. It is a fast and accurate sequence-based allergen prediction tool that models protein sequences as text
documents and employs support vector machine in text classification for allergen prediction [27]. Furthermore, for predicting the disorder among the amino acid sequences, DISOPRED [28] server was used.

3. Results

3.1 Sequence retrieval and identification of conserved regions
In total, VP1 capsid protein sequence of 92 different isolates of Enterovirus D68 found in the USA, Japan, China, New Zealand, France and the Philippines, was retrieved from NCBI GenBank sequence database (www.ncbi.nlm.nih.gov/genbank/) in FASTA format. To find the conserved regions, multiple sequence alignment (MSA) by CLUSTALW analysis in MEGA software was performed. CLUSTALW program generated several conserved sequences with varying lengths. The conserved sequences (length more than 15 amino acid residues) have been presented in Table 1.

A phylogenetic tree depicting the evolutionary divergence among the different VP1 proteins of EVD68 is presented in Figure 1.

Figure 1. A neighbor-joining tree showing the phylogenetic relationship among the VP1 protein of different isolates of Enterovirus D68.
3.2 Prediction of putative B cell epitopes from the conserved regions

On the basis of VaxiJen score (VaxiJen score ≥ 0.5) and transmembrane topology, among the conserved sequences, two regions “GLYGINPADTIGNLCVRIVNEHPQ” and “GLPDLTLQAMFVPTGALTP” were considered to be the most potential antigenic peptides which fulfilled the criteria of exomembrane characteristics as well (Table 1).

These two regions were then subjected to Bepipred [14] and ABCPred [15] analysis and subsequently “INPADT” epitope from “GLYGINPADTIGNLCVRIVNEHPQ” and “GALTP” from “GLPDLTLQAMFVPTGALTP” were found to be potential B cell epitopes.

To further strengthen the prediction, several additional tools from IEDB resource portal were employed. The surface accessibility of the peptides was determined by Emini surface accessibility prediction tool [17] using 1.000 threshold levels and one epitope “INPADT” was found to be above the threshold level (Table 2; Figure 2). The same epitope was also found to cross the threshold to be hydrophilic, flexible and have beta turn region determined by Parker hydrophilicity scale [18], Karplus and Schulz flexibility scale [19] and Chou and Fasman beta-turn prediction tool [20], respectively (Table 2; Figure 3, 4 and 5). However, “GALTP” epitope did not fulfill all the criteria.

The three dimensional structure of the capsid protein was obtained by SWISS-MODEL workspace [21] through automated homology modeling which is shown in Figure 6A and PROCHECK server validated the stereo chemical quality of the model through Ramachandran Plot (Figure 6B) [22], and QMEAN server [23] also assessed the tertiary structure, with a Qmean4 score of -2.54 (Figure 6C). The B cell epitope is marked by red color and it can be seen that the epitope is on the surface of the protein’s 3D structure hence proving its easy accessibility.

Figure 2. Surface accessibility of the conserved peptide. The surface accessible residues of the conserved peptide which are above the cut off value are located in the yellow region. The red horizontal line indicates surface accessibility cutoff (1.000).

Figure 3. Hydrophilicity of the conserved region. The hydrophilic residues are in the yellow colored region. The residues which are below the cut off (red line, 1.00) are in the green region.
Figure 4. Flexibility of the conserved region. The residues which are above the cut off value (1.00) are in the yellow colored region. The residues which are below the cut off (red line) are in the green region.

Figure 5. Beta turn prediction of the conserved region. The residues which are above the cut off value (1.0) are in the yellow colored region. The residues which are below the cut off (red line) are in the green region.

Table 1. Conserved sequences of VP1 capsid protein from EVD68 virus and their Vaxijen score, transmembrane topology and their position

<table>
<thead>
<tr>
<th>Conserved sequences of VP1 Capsid protein</th>
<th>Prediction of location using TMHMM server</th>
<th>Vaxijen value (Threshold =0.5)</th>
<th>Position of the selected sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>VTVRVYMKPKHIKAWAPRPPRTLPYMSIA NANY</td>
<td>Inside</td>
<td>0.5050</td>
<td>247-279</td>
</tr>
<tr>
<td>INQHGVSETLVENFLSRAALVSKRSFEYK</td>
<td>Outside</td>
<td>0.3313</td>
<td>61-89</td>
</tr>
<tr>
<td>SFVQLRRKLELFTYLFDRDAE</td>
<td>Inside</td>
<td>0.7124</td>
<td>111-130</td>
</tr>
<tr>
<td>GLYGINPADTIGNLCVRINNEHQP</td>
<td>Outside</td>
<td>0.8716</td>
<td>219-242</td>
</tr>
<tr>
<td>GLPDLTLQAMFVPTGALTP</td>
<td>Outside</td>
<td>0.9015</td>
<td>149-167</td>
</tr>
</tbody>
</table>

Table 2. Predicted common linear B cell epitope from Bepipred and ABCpred servers and the accessibility, hydrophilicity, flexibility and beta-turn prediction score for each residue

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Emini Surface accessibility score for each residue (threshold =1.00)</th>
<th>Parker Hydrophilicity Prediction score for each residue (threshold =1.00)</th>
<th>Flexibility Prediction score for each residue (threshold=1.00)</th>
<th>Beta-Turn Prediction score for each residue (threshold =1.00)</th>
</tr>
</thead>
<tbody>
<tr>
<td>INPADT</td>
<td>I 1.155</td>
<td>I -0.314</td>
<td>I 0.99</td>
<td>I 1.071</td>
</tr>
<tr>
<td></td>
<td>N 1.231</td>
<td>N 2.429</td>
<td>N 1.014</td>
<td>N 1.196</td>
</tr>
<tr>
<td></td>
<td>P 1.795</td>
<td>P 3.443</td>
<td>P 1.04</td>
<td>P 1.17</td>
</tr>
<tr>
<td></td>
<td>A 1.795</td>
<td>A 1.486</td>
<td>A 1.05</td>
<td>A 1.014</td>
</tr>
<tr>
<td></td>
<td>D 1.105</td>
<td>D 3.443</td>
<td>D 1.053</td>
<td>D 1.17</td>
</tr>
<tr>
<td></td>
<td>T 1.149</td>
<td>T 3.443</td>
<td>T 1.046</td>
<td>T 1.17</td>
</tr>
</tbody>
</table>
3.3. Prediction of T Cell Epitopes

T-cell epitopes were primarily selected by using the NetCTLv1.2 [24] server where the epitope prediction was confined to 12 MHC-I super types. The conserved sequences were also analyzed by a combined algorithm in IEDB analysis integrating MHC class I binding, TAP transport efficiency and proteasomal cleavage prediction (binding affinity kept within 100 nM) [25]. In total, 8 epitopes were found common in both analyses which are listed in Table 3.

These processed peptides were then analyzed by SMM based IEDB MHC I prediction tool and here again the cut off IC\textsubscript{50} value was set at 100nM. Thus, among these peptides, only those peptides which interacted with minimum four MHC class I alleles as well as were found to be overlapping with 15mers that interacted with a good number of MHC class II alleles, were selected finally (Table 4).

It was found “YMSIANANY”, “LVSKRSFEY”, “ENFLSRAAL” and “AMFVPTGAL”- these four epitopes were most likely to induce T cell immune response as they interacted with highest number of MHC class I and class II alleles.

3.4 Analysis of population coverage

IEDB population coverage tool [26] analyzed the population coverage of the proposed epitopes. The combined MHC-I and MHC-II class were assessed against the whole world population with the selected MHC-I and MHC-II alleles (Table 4). The epitope with the highest population coverage was found to be “LVSKRSFEY”.

3.5 Conservancy, allergenicity and disorder analysis

Since all the epitopes were predicted from conserved regions of VP1 protein they were 100% conserved among all the isolates found in various parts of the world.

In addition, it was found from Allerdictor server [27] all of the most suitable T cell and B cell epitopes were potentially non-allergen. Also, DISOPRED server [28] predicted the disorder of the conserved peptide “INQHGVSETLVENFLSRAALVSKRSFEYK” in order to get insight about the disorder among the sequence, which is depicted in Figure 7. It was found that the two most probable T cell epitopes- “LVSKRSFEY” and “ENFLSRAAL” lie outside of the disordered region to secure their potentiality as effective epitopes.

| Table 3. Common T-cell epitopes predicted by NetCTL server and Proteasomal cleavage/TAP transport/MHC class I combined predictor (IEDB AR) tool |
|---------------------------------|-----------------|-------------|----------------|
| Conserved Region                | T cell epitope  | Start Position | Interacting Super types |
| VTVRVYMKPKH1KAWAPRPPRTLPMYSIANANY | YMSIANANY   | 271        | A1,A26, B58,B62 |
| KAWAPRPPR                       |                 | 259        | A3            |
| GLYGINPADTIGNLCVRIVNEHQP         | DTIGNLCVR      | 227        | A26           |
| INQHGVSETLVENFLSRAALVSKRSFEYK   | LVSKRSFEY      | 80         | A1,A3, B58,B62 |
| NQHGVSFL                        | SETLVENFL      | 67         | B39,B44, B62  |
| ENFLSRAAL                       |                 | 72         | B39           |
| GLPDLTLQAMFVPTGALTP             | AMFVPTGAL      | 157        | A2, B62       |
**Table 4. Most probable predicted T cell epitopes obtained from conserved region interacting with different MHC class I and class II alleles**

<table>
<thead>
<tr>
<th>Most Putative T cell Epitopes</th>
<th>Interacting MHC-I alleles</th>
<th>Overlapping 15 mer peptides</th>
<th>Interacting MHC-II alleles</th>
<th>Population Coverage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>YMSIANANY</td>
<td>HLA-A*29:02</td>
<td>PPRTLPYMSIANANY</td>
<td>HLA-DRB1<em>01:01 HLA-DRB1</em>04:04</td>
<td>33.73</td>
</tr>
<tr>
<td></td>
<td>HLA-C*12:03</td>
<td></td>
<td>HLA-DRB1*04:04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HLA-B*15:01</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>HLA-B*15:02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HLA-C*03:03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HLA-C*14:02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVSKRSFEY</td>
<td>HLA-C*03:03</td>
<td>ENFLSRAALVSKRF</td>
<td>HLA-DRB1<em>01:01 HLA-DQA1</em>05:01</td>
<td>51.95</td>
</tr>
<tr>
<td></td>
<td>HLA-C*12:03</td>
<td></td>
<td>HLA-DQB1*03:01</td>
<td></td>
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<tr>
<td></td>
<td>HLA-B*35:01</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>HLA-A*29:02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENFLSRAAL</td>
<td>HLA-B*15:02</td>
<td>VENFLSRAALVSKRS</td>
<td>HLA-DRB1<em>01:01 HLA-DQA1</em>05:01</td>
<td>48.55</td>
</tr>
<tr>
<td></td>
<td>HLA-C*03:03</td>
<td></td>
<td>HLA-DQB1*03:01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HLA-C*12:03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HLA-C*14:02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMFVPTGAL</td>
<td>HLA-B*15:02</td>
<td>PDLTQAMFVPTGAL</td>
<td>HLA-DRB1*01:01</td>
<td>26.25</td>
</tr>
<tr>
<td></td>
<td>HLA-C*14:02</td>
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<td>HLA-C*12:03</td>
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<td>HLA-A*32:01</td>
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<td>HLA-C*03:03</td>
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### 4. Discussion

EV-D68 is now recognized as a reemerging pathogen. After the initial identification of EV-D68 in 1962, detection of this virus was rarely reported until the early 2000s. But recent studies have revealed that EV-D68 is detected among patients with acute respiratory infections of differing severities ranging from mild upper respiratory tract infections to severe pneumonia including fatal cases in pediatric and adult patients [29]. Despite the increasing epidemiological and clinical significance of this virus, till now there is no potential treatment to combat the infection. Thus, an effective cost effective prevention scheme would be most desirable for the public health providers as well as the scientific communities.

Vaccination has been one of the most important intervention techniques designed to prevent diseases that could be implemented on the affected population all over the world. In this study, an attempt was made to predict epitopes which could be tested for their efficacy in eliciting immunity through humoral and cell mediated immune responses. At present, vaccines are mostly based on B cell immunity. But recently, vaccine based on T cell epitope has been encouraged as the host can generate a strong immune response by CD8+ T cell against the infected cell [30].
Figure 6: A) Three-dimensional model of the VP1 protein with the proposed epitope in red. The outside location of the epitope indicates its surface accessibility. B) Ramachandran plot of the predicted model shows that most of the residues (83%) are in the allowed region of the plot, proving the validity of the model. C) QMEAN4 plot of the predicted model.

Figure 7: Protein disorder plot of "INQHGVSETLVENFLSRAALVSKRSFEYK" region. Amino acids in the input sequence are considered disordered when the blue line is above the grey dashed line (the confidence score is higher than 0.5). The orange line shows the confidence of disordered protein binding residue predictions.
With time, due to antigenic drift, any foreign particle can escape the antibody memory response; however, the T cell immune response often provides long-lasting immunity [31]. Therefore, in the present study both B cell and T cell epitopes were designed using \textit{in silico} computational approaches.

Inhibition of viruses at the stage of viral attachment provides a target for therapeutic intervention [32]. In order to predict a peptide that can be used in vaccine development to prevent viral entry or its interaction with host cell, this study focused on VP1 capsid protein. This protein is required by enterovirus for mediating viral adsorption and uncoating process [33] and subsequently becomes a good target for vaccine designing.

Here, multiple prediction methods were applied to determine a potential B-cell epitope considering several criteria like hydrophilicity, surface accessibility, flexibility and beta-turn. The proposed epitope “INPADT” has met all the criteria of the above B-cell prediction methods. Furthermore, the three-dimensional model of the capsid protein ensured the exact location of the epitope outside of the protein surface and the model validity was evaluated by Ramachandran Plot, where it was seen 83 % amino acid residues were within the favored region.

Along with the B-cell epitope, in this study, T-cell epitopes were also designed. Due to lack of a consensus mapping protocol with immunoinformatics tools, a combined prediction method was applied according to the hypothesis of Trost et al. [34] who proposed that greater prediction accuracy can be achieved by combining the predictions from several algorithms rather than relying on just one. Both NetCTL and IEDB analysis tools were used to find epitopes for the activation of T-cell immunity with potential antigenicity. By examining the output it was predicted that “YMSIANANY”, “LVSKRSFGEY”, “ENFLSRAAL” and “AMFVPTGAL”- these four epitopes would be the best epitope candidates. They interacted with the highest numbers of MHC class I alleles, were the core peptides of a good number of MHC class II binding predictions and also demonstrated large world population coverage. Again, conservancy is another very important criterion of an epitope to consider it for vaccine development and all the proposed epitopes were 100% conserved since they were predicted from conserved region analysis.

However, allergenicity is one of the prominent obstacles in vaccine development since today most vaccines stimulate the immune system into an “allergic” reaction, [35] through induction of type-2 T helper (Th2) cells and immunoglobulin E (IgE). \textit{In silico} analysis revealed that all the vaccine candidates predicted in this work were non-allergenic in nature.

Thus, it is believed that these suggested B and T cell epitopes will definitely reduce time, cost and labor during \textit{in vivo} and \textit{in vitro} studies to be carried out for developing a vaccine against Enterovirus D-68.

\textbf{Conclusion}

In this study, an attempt was made to design epitope based vaccines against Enterovirus D68 which could be tested for their efficacy in eliciting immunity through humoral and cell mediated immune responses. The results are based on sequence analysis and computational predictions. However, to prove the effectiveness of mounting an immune response, both \textit{in vitro} and \textit{in vivo} studies are required along with this \textit{in silico} study.

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