In Silico Prediction of Human Parechovirus Epitope-Based Vaccine Candidates

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ABSTRACT

Human parechoviruses (HPeVs) have emerged globally as a potential cause of severe life-threatening illness among neonates and young children. HPeVs, consisting of 17 genotypes causing differential clinical diseases. The most prevalent are HPeV1 and HPeV3. HPeV1 causes primary gastrointestinal infections. Moreover, HPeV3 causes sepsis and disorders of the central nervous system (CNS) in young infants. Although the mortality rate by HPeV infection is rare, it may cause severe neurodevelopmental sequelae. HPeV-3 has been described as the most commonly detected type of HPeV in cerebrospinal fluid (CSF) from hospitalized children. HPeV3 is also a common single cause of aseptic meningitis/meningoencephalitis in infants under 90 days of age, usually seasonal with summer fall. The infection of CNS with HPeV3 usually lack pleocytosis of the CSF. Comparative analysis of HPeV's genomes will help to identify the core characteristics that characterize this virus family's unique properties. HPeV, capsid polyprotein along with the RGD motif, was acquired from a protein database, and we anticipated the most immunogenic epitope for cell-mediated immunity T cells and B cells. FLNFKSMNV, KVFENSYSY, KTKYLTMSTK, SVYASTFNR were the most potent peptides for CD4+, and CD8+ T cells predicted as epitopes. However, EVLNRLTYNY and FAYFTGELNI had the most impressive pMHC-I immunogenicity score and were pursued for their association with HLA particles using silico docking systems to validate the binding cleft epitope. For future planning of an epitope-based peptide immunization against HPeV, we assume this model will help to create and anticipate potential contender for the antibody advancement. In contrast, a phylogenetic analysis may provide details about evolutionary relationships and protein ancestry.

Keywords: Human parechoviruses (HPeVs), RGD motif, Epitopes, Immunogenomics, *Insilico*, Vaccine development, MHC class

INTRODUCTION

First isolated in 1956, Human parechoviruses (HPeVs), classified as entHPeV infections, account for the high proportion of sepsis-like illnesses in infants under < 90 days. Earlier were named eroviruses (Echoviruses 22 and 23), but in 1997 genus Parechovirus was assigned separately[1]. These infections are uncommon in older children and adults, although they often manifest as gastrointestinal or respiratory infections in young children[2]. HPeV infection has been recognized as infant clusters and outbreaks[3,4]. Infants infected may exhibit symptoms similar to sepsis, which can affect the central nervous system (CNS). These symptoms are difficult to differentiate from those of bacterial sepsis through clinical observation[5,6]. CSF inflammatory indicators are somewhat elevated, and there is limited pleocytosis, yet they may present with severe neurological impairment or convulsions[7]. Severe HPeV infections in infants are associated with long-term complications[6-8]. However, HPeV type 3 (HPeV3) creates severe systemic illnesses in young infants. It is strongly advised to conduct tests for enteroviruses (EV) and HPeV in all neonates with sepsis-like symptoms due to the unpredictable clinical presentation of the disease.

Enhancing the understanding of Human parechovirus (HPeV) virology and the distinct protein mechanisms involved in the viral replication cycle can be advantageous in identifying novel targets for antiviral therapy. Managing severe HPeV infection includes early diagnosis and excellent support care to decrease complications. To date, no antiviral drug is useful in the treatment of HPeV infections; currently, no effective anti-infection vaccines are available. Severe HPeV disease is treated with Intravenous Immunoglobulins (IVIGs). However, there are apprehensions about IVIG being effective against all HPeV genotypes[9]. Whereas, there have been few types of research done on the specific therapy against HPeV infections. Antivirals such as Pleconaril and itraconazole, Pleconaril, and brefeldin A have only been found effective in vitro[9]. Currently, there is no vaccine or commercially available treatment against HPeV, regardless of the CNS infections and sepsis-like diseases occurring increasingly in clusters distributed among different regions worldwide. The present investigation employed diverse computational methodologies to identify B-cell and T-cell epitopes from Capsid polyprotein, which may serve as a promising vaccine candidate against HPeV. Four distinct regions make up the HPeV genome: 5 = untranslated region (UTR), 1 = open reading frame (ORF), 3 = UTR, and a poly (A) tract (Figure 1).

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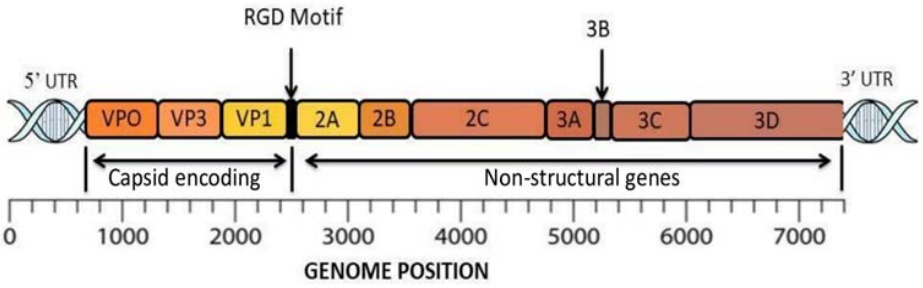

Figure 1: Human parechovirus genome organization (Re-created from Ruoslahti, 1996)

A single ORF converts all structural and non-structural proteins into one polyprotein, further divided into multiple precursor molecules[9]. The N-terminal portion of the polyprotein contains the capsid structural proteins VP0, VP1, and VP3, as well as non-structural proteins 2A, 2B, 2C, 3A, 3B, 3C, and 3D[10]. The RGD motif of the VP1 protein was shown to be a crucial immunogenic epitope in previous investigations of acquired immune responses[11]. VP0 protein has been identified as antigenic in some picornaviruses as another antigenic site. Several studies showed that all three capsid proteins (VP0, VP1, and VP3) contain antigenic locations[12,13]. Shakeel et al. (2015) use human donor antibody-producing B-cells to generate two distinct monoclonal antibodies: AM18 and AM28, each specific to HPeV-1[14]. The VP1 protein, including the RGD motif, has the AM18 epitope and may work by making virus aggregations neutralize them and preventing integrin binding to the capsid virus. In contrast, the AM28 will likely inhibit RNA uncoating by recognizing a conformational epitope on VP0 and VP3 loops from adjacent capsid-based pentamers[14,15]. In this study, authors have employed various computational techniques to detect B-cell and T-cell epitopes of capsid protein, which could serve as a basis for developing a future HPeV vaccine.

MATERIALS AND METHODS

Selection of Potential Candidates for Vaccine Development: The candidate vaccine was constructed by retrieving the different structural protein sequences of the HPeV virus from UniProt. A polyprotein of capsid proteins (VP0, VP1, VP3 and VP3 c-terminal, including RGD motif) was selected. Capsid proteins help viruses in host invasion and the assembly of viral particles[11,13,14].

Retrieval of Protein Sequence: To cover maximum outbreaks from the past and taking into account their dates, all 19 available sequences of HPeV structural proteins were retrieved form NCBI database in FASTA format.

Analysis of Structural Proteins Variability: The level of conservation among retrieved sequences was analyzed. A multiple sequence alignment (MSA) was acquired using the Omega program EBI-Clustal[16].

Prediction of the Structural protein Antigenicity: Developing a peptide vaccine to identify the protein candidates that display antigenic features is essential. The designated sequence's antigenicity was evaluated by applying the Kolaskar & Tongaonkar method[17] and the VaxiJen v2.0 server[18]. A reference human parechovirus 2, with accession number 39085, was tested for testing antigenicity.

Prediction of Linear B-cell Epitope: To ensure accurate B cell epitope prediction, we utilized the BepiPred 2.0[17] and ABCpred[18], web servers[19], thereby minimizing the occurrence of false-positive outcomes. The sequence of interest was subjected to epitope prediction using BepiPred 2.0[17], a computational tool that utilizes propensity scale and a hidden Markov model. While in the ABCpred server, parameters, such as the window length of 12 amino acids and the threshold at seventy five percent, were set[18]. All the frequently predicted epitopes using both servers were examined and chosen for further evaluation.

Surface Accessible Regions Prediction: While access to a cell surface receptor, or an antibody, an optimal epitope causes an immunogenic reaction[20]. To assess the surface accessibility of the epitope, the complete set of queries was submitted to the Emini Surface Accessible Prediction Tool (IEDB)[21]. B cell epitopes were selected after comparing them with the resulting accessible regions.

B-cell Epitope Conservancy Analysis: An epitope is ideal when conserved and can elicit an immune response against various strains and occasionally among species. Selected epitopes/glycoprotein sequences were submitted to IEDB's Epitope conservation tool for analyzing conservancy[22]. A threshold of 80% was set for sequence identity while predicting Conservancy.

B-cell Epitopes Antigenicity Prediction: The VaxiJen v2.0 system[23] was used to test the antigenicity of the chosen B cell epitopes. This tool, an independent alignment approach, predicts peptide antigenicity in amino acids based on physicochemical characteristics.

B-cell Epitopes flexibility and Hydrophilicity Prediction: The antigenicity of peptides is primarily associated with their hydrophilicity and flexibility[24]. The Parker hydrophilicity prediction tool and the Karplus and Schulz (KS) method were utilized to assess these properties. The epitopes that were conserved were chosen by considering their anticipated hydrophilicity and flexibility[25-27]. The Karplus and Schulz (KS) approach is a commonly used tool to analyze proteins' flexibility. Standardized B-values of Ca-atoms in protein structures are used for flexibility prediction[28-30].

T-cell Epitope Prediction and Analysis of Conservancy: T cell epitopes were discovered using the NetCTL server at the IEDB[17]. A cut-off of 0.50 was established, with a specificity of 0.94 and a sensitivity of 0.89. In an integrated algorithm, NetCTL predicts T cell epitopes by considering the class I affinity of MHC, the proteasomal cleavage of TAP, and the transportation efficiency of TAP. The epitopes with the highest combined scores were chosen for analysis, resulting in a selection of seven epitopes. To learn how specific MHC-I alleles interact with certain epitopes, we used the IEDB's MHC-I prediction website[31]. The determination of the half-maximum inhibitory concentration (IC50) of peptides interacting with MHC-I alleles was achieved through the utilization of the Stabilized Matrix Method (SMM), as reported in reference[31]. A cut-off IC_{50} value of 200 nM was determined. The analysis included all possible MHC class I alleles. Peptides were limited to a maximum of nine amino acids in length.

Additionally, the MHC class II interaction was determined using the IEDB MHC class II binding prediction program[32]. Nonetheless, entire protein sequences were utilized because MHC class II can accommodate significantly longer peptides and, on occasion, complete proteins. IC50 values for chosen epitopes binding to MHC II molecules were determined using the Stabilized Matrix Base Method (SMM). The SMM-align technique was utilized for predicting appropriate binders, with a cut-off value of IC50 set at 100 nM. Epitopes were selected again using the interaction with the most significant number of alleles. The epitopes that exhibit binding predictions between MHC I and MHC II and interact with the vital number of alleles, with a minimum of three alleles, were chosen to anticipate population coverage and epitope conservancy, as per previous research[33]. Before submitting the chosen T-cell epitopes, the IEDB conservancy analysis toolset a threshold of 80% for protection[22].

T-cell Epitope Allergenicity Analysis: A prerequisite for vaccine development is the absence of any allergic response in the ideal candidate. To assess the allergenicity of T-cell epitopes, we use AllerTOP v.2. An alignment tool, AllerTOP, predicts allergens *in silico* based on the physicochemical properties of proteins. AllerTOP exhibits a high level of sensitivity (94%) compared to alternative tools[34]. To further confirm the outcomes, we use Allergen FP software. This program uses the amino acid's size, quantity, hydrophobicity, and tendency to form β-strands and helices as predictors of allergenicity[35].

T-cell Epitopes Toxicity Analysis: The success of peptide-based therapy can be hampered by many factors, among which the most significant is Toxicity. Preferably, an epitope should have high antigenicity with no or slightest toxicity[36].

Analysis of T-cell Epitope Coverage of the Global Population: Coverage Analysis Tools for the population determine what proportion will respond to predetermined epitopes based on their shared MHC restrictions. For a high-quality vaccine contender, it is never adequate to predict T-cell epitopes alone; it must have sufficient human population coverage in most areas. Each selected-cell epitope and its associated MHC class I and II alleles were analyzed for global population coverage using the population coverage analysis tool of IEDB. All parameters were left at their default settings for the submitted sequence analyses. This tool performs the following analyses and computations for calculating population coverage: First, the proportion of the population that would be protected; second, the average number of epitope hits per HLA combination; and third, the total number of HLA / epitope hits recognized by 90% of the population (PC90). Due to the lack of linkage equilibrium between HLA loci[22], these estimates must rely on genotypical HLA frequencies.

T-cell epitopes (conserved) and selected HLA-B*53:01 and HLA-A*26:01 3D structure prediction

Target Preparation: We retrieved the HLA-B*53:01 (PDB; ID: 1A1M) three-dimensional structure from the Protein Data Bank (PDB) (http://www.rcsb.org/pdb/home/home.do) and for allele HLA-A*26:01 (3d design not available), we have predicted structure from Phyre2 server (PMID: 25950237) and validated by SAVES server (PMID: 8950272). For the crystal structure of these alleles, the swiss_PDB viewer tool removed the water molecule and added the missing hydrogen atoms, minimizing the energy level[37].

Ligand Preparation: The peptide structure was predicted using the PepFold server (http:/bioserv.rpbs.univ-paris-dideroth.fr / PEP-FOLD/), while peptide geometry was optimized using the ChemBio3DUltra12 software (PerkinElm Informatics, Waltham, MA, USA). Both peptides were stored in PDB[38].

Molecular Docking and Molecular Dynamics study of T-cell epitopes with the HLA-B*53:01 and HLA-A*26:01

Docking studies revealed crucial information about inhibitor-binding pocket orientation. Most docking techniques in this work are employed primarily for screening tiny, drug-like compounds[39], but their selectivity and efficiency dropped dramatically for ligands with more than ten flexible bonds. To overcome this limitation, an online server called Dinc 2.0 was used to predict the binding modes of large ligands like peptides. Dinc 2.0 selected EVLNRLTYNY and FAYFTGELNI as the first docking inputs with the receptor's HLA-A*26:01 and HLA-B*53:01, respectively. Multiple parallel docking runs chose the best binding modes, and many atoms were added to extend the corresponding fragments. These extended fragments were used as inputs for the second round of docking (DoFs) to explore their new subset of flexible degrees of freedom. These flexible DoFs represented, as shown in the best possible binding mode, some "new" (red) and some "old" (blue) atoms. This process continued until the entire ligand (bottom left and right) had been reconstructed[40]. In order to display the docking interaction, the PYMOL molecular graph TOICS method, Version 1.7.4.4, was used[41].

RESULTS

Structural protein in most pathogenic HPeV strains is conserved

Capsid protein among 19 strains was found to be mostly conserved. Protein Variability Server[42] showed that 15 nucleotides were fully conserved, comprising >95% of the sequence of Capsid protein (Figure 2).

Antigenicity of Capsid Protein: A peptide or protein as a vaccine candidate should be enough antigenic to incite an adequate immune response. The potential antigenic properties of a Human Parechovirus 2 strain, identified by accession number 39085, were assessed using the VaxiJen server. The strain was found to have a value of 0.4949, indicating its potential as an antigen. The threshold was set as 0.4 for the antigenicity of the virus. With a window size of seven amino acids, we utilized the Kolaskar & Tongaonkar antigenicity prediction tool to assess the core amino acid antigenicity for each residue in the capsid protein[23,43]. The cut-off value was more than 1.00 for most amino acids across all 19 protein sequences (Figure 3). Position 12 residues had a minimum value of -0.006 and a maximum score of 2.158, with an average score of 0.186.

All prediction tools recognized TTSEPENKDVVQ, PHVLMNLA-ETTQADLC, TAGSKSRFD, and YFKWSANSDPQAIVHRNLVH as B-cell epitopes.

BepiPred and ABCpred were used to predict 10 and 8 peptides, respectively, as B cell epitopes. Antigenic epitopes were considered for further investigation as indicated by both tools. We found that four epitopes were shared among all prediction tools when further analyzed (Table 1).

Surface accessibility property was found in QATTTVNTTN, PHVLMNLAET, PHVLMNLAETTQADLC, and QVNVNQGTAG, respectively. Emini Surface Accessibility prediction approach[44] was used at threshold cut-off 1.0 during the analysis of the capsid protein's surface accessibility properties. The length of the identified four peptides having scored above the threshold were 6,12, 8, and 11 amino acids, respectively. These peptide sequences are TVNTTN, TYATTSEPENKD, TNLTQHPS, MSTKYKWTRNK, respectively. The selected epitopes and the four peptides were compared (Table 2 and Figure 4).

Table 1: Commonly recognized B-cell epitopes by ABCPRED and BEPIPRED 2.0

S. No.	Sequences	Start	End	Length
1	QVNVNQGTAG	172	182	10
2	GSMNMANVLS	323	333	10
3	QATTTVNTTN	70	80	10
4	PHVLMNLAET	208	218	10
5	YATTSEPENKDVVQATTTVNTTNLTQHPSA	57	85	30
6	PHVLMNLAETTQADLC	208	223	16
7	KSIADMATGVTKTIDATINSVNEIITNTDNASGGDILTKVADDASN	32	50	46
8	YLTMSTKYKWTRNKVDIAEGPGSMNMANVLSTTAAQSVALV	320	340	41

Table 2: Emini surface accessibility prediction analysis predicted the following surface-accessible antigenic sites

S. No.	Peptide	Position	Length
1	TVNTTN	25-30	6
2	TYATTSEPENKD	40-51	12
3	TNLTQHPS	62-69	8
4	MSTKYKWTRNK	136-146	11

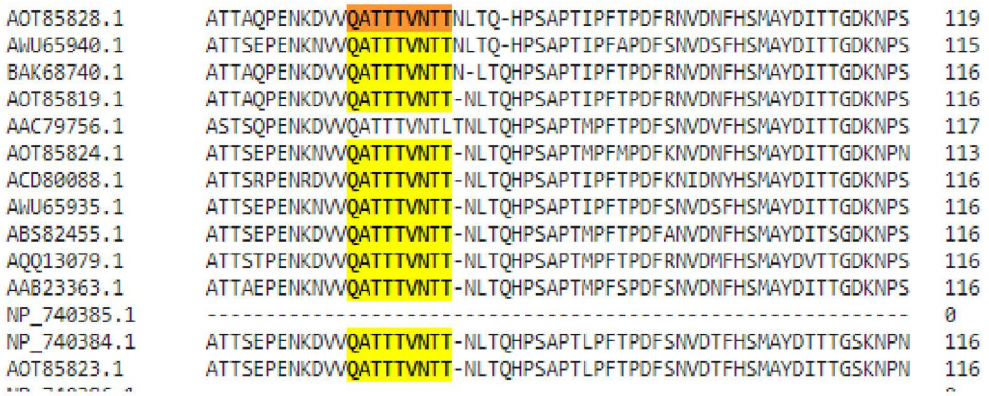

Figure 2: Jalview Image of the MSA of all the 19 available sequences of protein

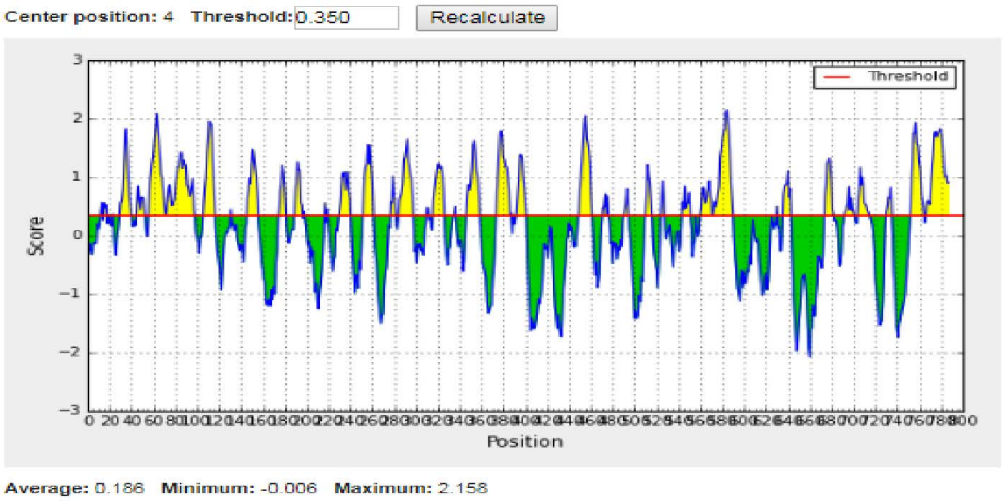

Figure 3: High antigenic nature of capsid protein is indicated by the yellow regions (1.00 is the threshold here)

All selected epitopes showed consensus when compared with these three predicted surface-accessible peptides. Thus, indicated that all these epitopes and Emini-predicted peptides overlap, i.e., they are harbored in the same vicinity of capsid protein. They were QATTTVNTTN, PHVLMNLAET, PHVLMNLAETTQADLC, and QVNVNQGTAG. Further conservancy analysis of these selected four epitopes was done.

Among all sequences, QATTTVNTTN, PHVLMNLAET, PHVLMNLAETTQADLC, and QVNVNQGTAG were highly similar

Compared with highly variable genomic regions, conserved epitopes give more extensive protection over various strains or even species. Along these lines, in an epitope-based immunization advancement, a perfect epitope ought to be very similar or conserved. Using the IEDB conservancy analysis tool, all four B cell epitopes were evaluated for their conservancies[22]. High similarity was identified for the selected epitopes among all sequences of capsid protein (Table 3).

Highly antigenic sequences found were QATTTVNTTN, PHVLMNLAET, PHVLMNLAETTQADLC, and QVNVNQGTAG. VaxiJen server (Doytchinova and Flower, 2007) recognized QATTTVNTTN, PHVLMNLAET, PHVLMNLAETTQADLC, and QVNVNQGTAG as likely antigens with values of 0.9165, 0.4373, 0.5576, and 0.6817, respectively. The default value of 0.4 was used for the antigenicity cut-off.

The nature of QATTTVNTTN is highly hydrophilic and flexible

Two vital characteristics of an epitope are flexibility and accessibility to induce effective immune reaction[24]. Flexibility prediction analysis of all highly similar epitopes by Karplus and Schulz showed that QATTTVNTTN was highly flexible[45] (Figure 5).

In order to evaluate the QATTTVNTTN hydrophilicity, IEDB Parker hydrophilicity analysis was then conducted[25]. Seven amino acids were used for the window size, with four in the center (Table 4 and Figure 6).

T-cell Epitopes and Conservation Prediction: NetCTL server predicted several potential T cell epitopes[46] within selected parameter settings; however, based on the high Conservancy and combinatorial

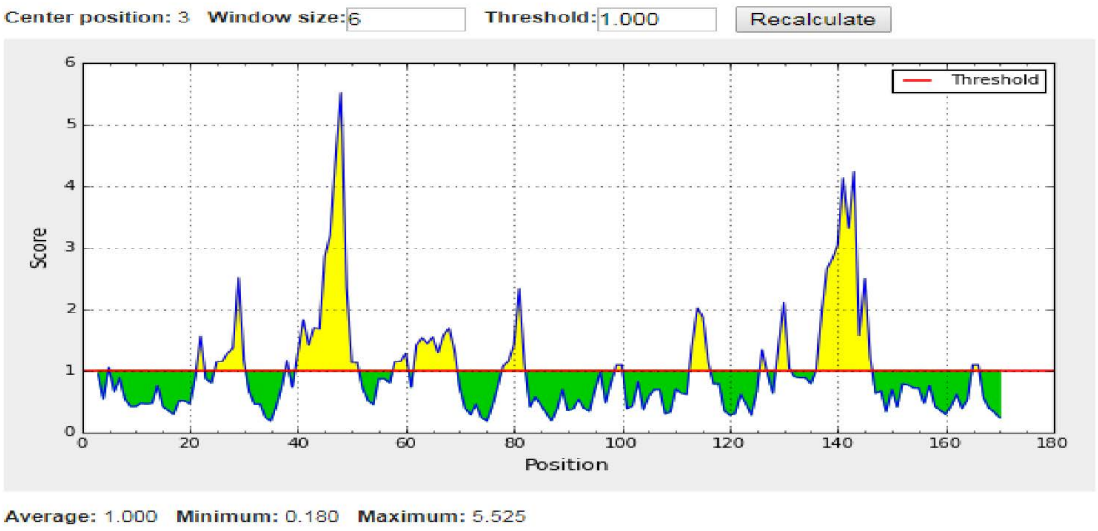

Figure 4: Surface accessibility of capsid protein. The surface accessibility cut-off is represented with the horizontal red line, whereas surface accessible epitopes are indicated with the yellow regions above the red line

Table 3: IEDB conservancy analysis showed the consensus sequences between the Emini surface peptides and predicted B cell epitopes as follows

S. No.	Sequence	Length	Percent of protein sequence matches at identity ≤ 100%	Minimum identity	Maximum identity
1	QVNVNQGTAG	10	60.00% (9/15)	30.00%	100.00%
2	GSMNMANVLS	10	40.00% (6/15)	40.00%	100.00%
3	QATTTVNTTN	10	80.00% (12/15)	30.00%	100.00%
4	PHVLMNLAET	10	86.67% (13/15)	30.00%	100.00%
5	YATTSEPENK DVVQATTTVN TTNLTQHPSA	30	80.00% (12/15)	30.00%	100.00%
6	PHVLMNLAET TQADLC	16	86.67% (13/15)	18.75%	100.00%
7	KSIADMATGVTKTID ATINSVNEIITNTDNAS GGDILTKVADDASN	46	6.67% (1/15)	17.39%	100.00%
8	YLTMSTKYKWTRN KVDIAEGPGSMNM ANVLSTTAAQSVALV	41	6.67% (1/15)	17.07%	100.00%

Table 4: Predicted peptides with high flexibility and values above threshold of 1.087

S. No.	Sequences	Position	Length	Score
1	QATTTVN	1-7	7	1.051
2	ATTTVNT	2-8	7	1.037
3	TTTVNTT	3-9	7	1.032

Table 5: 7 epitopes were selected from NetCTL based on the high combinatorial scores and their Conservancy

S. No.	Epitome sequences	Amino acid positions	Threshold	Supertype	% protein sequence matches at identity ≤ 100%	Minimum identity	Maximum identity
1	FLNFKSMNV	93-101	0.1	A1	53.33% (2/15)	33.33%	100.00%
2	KVFENSYSY	503-511	1.0415	A1	13.33% (2/15)	33.33%	100.00%
3	SVYASTFNR	457-456	3.2686	A1	33.33% (5/15)	33.33%	100.00%
4	EVLNRLTYNY	432-440	1.5170	B58	80.00% (12/15)	30.00%	100.00%
5	KTKYLTMSTK	423-54	1.4326	A24	6.67% (1/15)	30.00%	100.00%
6	SVYASTFNR	403-411	1.5461	B39	33.33% (5/15)	33.33%	100.00%
7	FAYFTGELNI	533-541	1.2913	B7	86.67% (13/15)	30.00%	100.00%

Table 6: Predicted T-cell epitopes with their interacting MHC-I alleles

Epitopes	MHC-I Alleles	IC_{50} (nM)	Epitopes	MHC-I Alleles	IC_{50} (nM)
FLNFKSMNV	HLAA*02:01	0.560	EVLNRLTYNY	HLA-A*26:01	14.14
	HLA-A*02:03	15.85		HLA-B*53:01	3.5
	HLA-A*02:06	34.83		HLA-A*30:02	22
				HLA-B*57:01	26
				HLA-A*01:01	2.8
				HLA-A*68:01	368.97
				HLA-A*23:01	68.94
				HLA-A*32:01	22
				HLA-A*31:01	91.75
KVFENSYSY	HLAA*30:02	47.26	KTKYLTMSTK	HLA-A*03:01	2.4
	HLA-A*32:01	5.14		HLA-A*11:01	0.2
				HLA-A*30:01	178.21
				HLA-B*57:01	16
				HLA-A*31:01	1.5
SVYASTFNR	HLA-A*31:01	70.86	FAYFTGELNI	HLA-B*53:01	243.13
	HLA-A*03:01	0.15		HLA-A*23:01	0.69 2.
	HLA-A*68:01	72.11		HLA-A*24:02	17.55
	HLA-A*11:01	212.74		HLA-A*02:01	1.8
	HLA-A*33:01	187.09		HLA-A*02:06	5.4
				HLA-B*58:01	9.5
				HLA-B*51:01	0.04

Table 7: Seven shortlisted T-cell overlapping epitopes between MHC I and MHC II binding predictions

Epitopes	MHC- I Alleles	MHC-II Alleles	Peptide Sequences
FLNFKSMNV	HLAA*02:01	HLA-DRB1*15:01	NDKPNYFLNFKSMNV
	HLA-A*02:03	HLA-DRB1*07:01	NDKPNYFLNFKSMNV
	HLA-A*02:06		KPNYFLNFKSMNVDI
KVFENSYSY	HLAA*30:02	HLA-DRB1*07:01	KVFENSYSYFRGSLI
	HLA-A*32:01	HLA-DRB4*01:01	LKVFENSYSYFRGSL
		DRB5*01:01	FPNLKVFENSYSYFR
		HLA-DRB5*01:01	PNLKVFENSYSYFRG
			LKVFENSYSYFRGSL
			KVFENSYSYFRGSLI
SVYASTFNR	HLA-A*31:01	HLA-DRB5*01:01	LSVYASTFNRGRLNG
	HLA-A*03:01		RLSVYASTFNRGRLN
			IRLSVYASTFNRGRL
	HLA-A*68:01	HLA-DRB5*01:01	LSVYASTFNRGRLNG
	HLA-A*11:01		RLSVYASTFNRGRLN
	HLA-A*33:01		IRLSVYASTFNRGRL
		HLA-DRB1*07:01	SLIIRLSVYASTFNR

EVLNRLTYNY	HLA-A*26:01 HLA-B*53:01 HLA-A*30:02 HLA-B*57:01 HLA-A*01:01 HLA-A*68:01 HLA-A*23:01 HLA-A*32:01 HLA-A*31:01	HLA-DRB1*03:01	GLFQIEVLNRLTYNY LFQIEVLNRLTYNYS
KTKYLTMSTK	HLA-A*03:01 HLA-A*11:01 HLA-A*30:01 HLA-B*57:01 HLA-A*31:01	HLA-DRB5*01:01	PLRKTKYLTMSTKYK KTKYLTMSTKYKWTR LRKTKYLTMSTKYKW RKTKYLTMSTKYKWT
FAYFTGELNI	HLA-B*53:01 HLA-A*23:01 HLA-A*24:02 HLA-A*02:01 HLA-A*02:06 HLA-B*58:01 HLA-B*51:01	HLA-DRB1*15:01	SLLFAYFTGELNIHV
		HLA-DRB3*02:02	IEFAYFTGELNI SSR

Table 8: Combined prediction of linear B-cell epitope showed the amino acid residues within the region from 57 to 85 are having antigenic propensity highest for the B-cell linear epitopes within HPEv capsid polyprotein

Method	Region	Residues	Score Avg.	Min.	Max.
Emini Surface Accessibility Prediction	73-79	TVNTTN	11.000	0.269	3.338
Kolaskar & Tongaonkar Antigenicity	68-75	VVQATTT	0.997	0.907	1.082
Karplus & Schulz Flexibility Prediction	59-65	TTSEPEN	1.037	0.972	1.097
Parker Hydrophilicity Prediction	73-79	TTVNTTN	4.057	3.743	4.443
Bepipred Linear Epitope Prediction n	70-79	QATTTVNTTN	0.969	0.823	1.102
Chou & Fasman Beta-Turn Prediction	61-67	SEPENKD	1.005	0.789	1.209

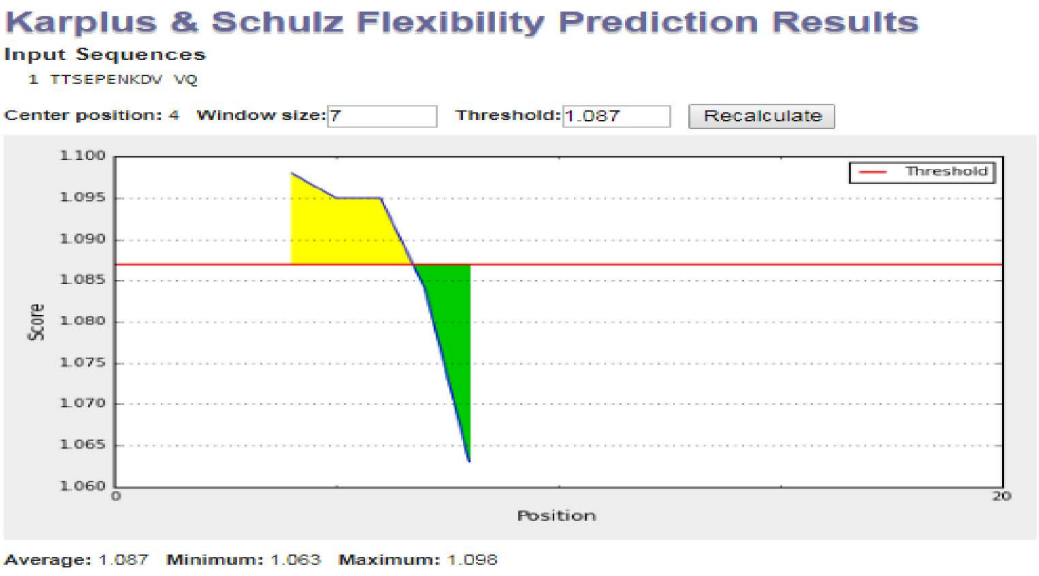

Figure 5: QATTTVNTTN epitope flexibility. This epitope's amino acids were found to be above the threshold level

scores among the available strains, only seven epitopes were selected (Table 5). With these selected seven epitopes, we found reacting few MHC-I class alleles identified using the MHC-I binding prediction server. 200 was set as an IC50 cut-off value[31,47] (Table 6). The whole capsid protein sequence was also submitted to the binding prediction tool IEDB MHC class II for analysis[32] (Table 7).

Selection of non- toxic and non-allergens T-cell epitopes

Toxicity data for the selected T-cell epitopes was examined by the ToxinPred service and 7 T-cell epitopes were predicted to be safe using AllerTOP v 2.0[34] and AllergenFP[48].

Selection of T-cell epitopes with the highest world population coverages

The seven selected epitopes were examined using the IEDB population coverage program. The two epitopes with the highest population coverage were EVLNRLTYNY and FAYFTGELNI (Figure 7).

Population coverage for most likely epitopes varied from 6% to 75% when both MHC class I and class II alleles were taken into account. (Table 8). World Population coverage of EVLNRLTYNY and FAYFTGELNI was found to be 75% and 70%, respectively. The PEPFOLD server was used to generate 3D models from the two selected epitopes. The IEDB Population Coverage Analysis Tool[22] analyzed the seven selected T-cell epitopes for their worldwide coverage.

Prediction of 3D structures and Molecular docking for epitopes EVLNRLTYNY and FAYFTGELNI

Figure 8a and 8b represent the predicted 3D Structure of EVLNRLTYNY and FAYFTGELNI, respectively. Figure 9A represent the peptide FAYFTGELNIS binding in HLA-B*53:01 groove. Figure 9B represent the molecular docking results with lowest energy poses in HLA-A*26:01 binding site for EVLNRLTYNY.

Figure 10 represent the predicted 3D structure of the HLA-A*26:01 allele and show the molecular docking results of HLA-A*26:01 with EVLNRLTYNY. The Ramachandran plot of HLA-A*26:01 shows the model's G-factor in addition to statistics indicating residues in the most favorable and disallowed regions.

DISCUSSION

Human parvoviruses (HPeVs) are members of the Parechovirus genus of the Picornaviridae family and are responsible for a wide variety of human enterovirus-related illnesses[12,49-52]. In Japan[53,54], Thailand[55]. Canada[56,57], the Netherlands[8,58,59], Norway[60], Scotland[61,62], Australia[63], Germany[64], Spain[61,65] and the United States[66], HPeV3 has been reported in conjunction with meningitis, gastroenteritis, respiratory disease, severe neonatal viral sepsis, and transient paralysis. Human parechoviruses (HPeVs) are causing 3 million annual infections. The virus has changed from obscure to turning into a global risk to general well-being that influences a considerable number of kids worldwide.

Table 9: Results from epitope docking (EVLNRLTYNY and FAYFTGELNI) with HLA-A*26:01 and HLA-B*53:01 molecules

Protein	Peptide	Energy (kcal/mol)	Inhibition Constant (nM)	Contact residues	Surrounding residues
HLA-A*26:01	EVLNRLTYNY	-6.10	3.37782E-05	MET-1, THR-8, SER-26, HIS-27, ASP-53, ASP-54, GLN-56, GLU-236	ALA-2, VAL-3, ALA-5, PRO-6, ARG-7, LEU-9, ALA-24, SER-28, ASP-126, VAL-127, ARG-135, LEU-196, LEU-156, GLN-204, THR-206, ALA-235, GLY-253, PRO-259
HLA-B*53:01	FAYFTGELNI	-8.50	5.88047E-07	SER-1, ARG-6, TYR-27, ASP-29, GLN-32	GLY-1, SER-2, HIS-3, VAL-28, ASP-30, THR-178, LEU-179, TYR-209, PRO-210, GLU-212, PRO-235, PHE-241

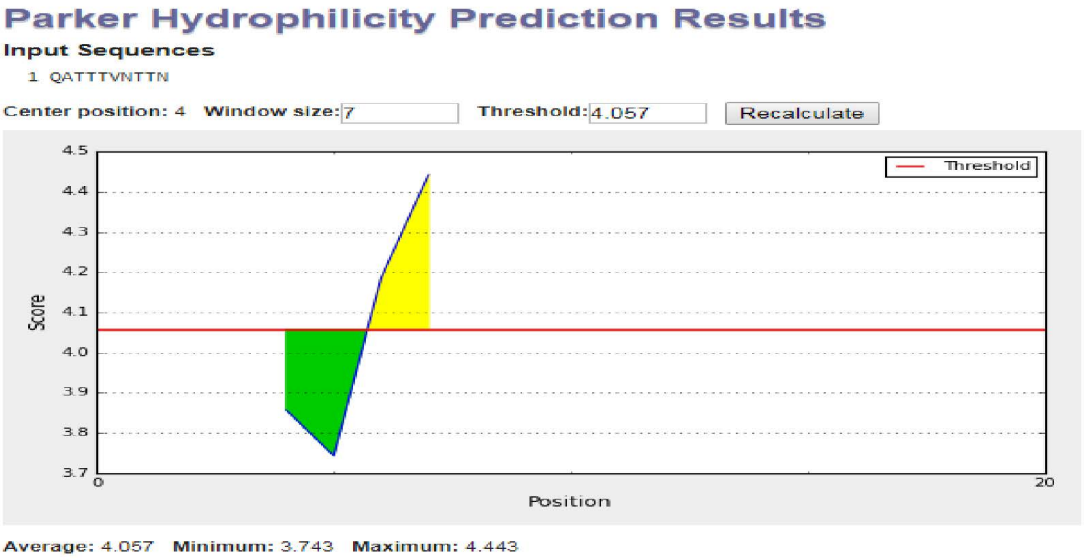

Figure 6: QATTTVNTTN epitope hydrophilicity. Many of the residues of this chosen epitope were hydrophilic. Residues above cut-off 3.743 (horizontal red line) are in the yellow area

Population Coverage Calculation Result

population/area	Class I		
	coverage[a]	average_hit[b]	pc90[c]
World	81.5%	1.39	0.54
Average	**81.5**	**1.39**	**0.54**
Standard deviation	**0.0**	**0.0**	**0.0**

[a] projected population coverage
[b] average number of epitope hits / HLA combinations recognized by the population
[c] minimum number of epitope hits / HLA combinations recognized by 90% of the population

Population: World

MHC class	Coverage	Average hit	PC90
I	81.5%	1.39	0.54

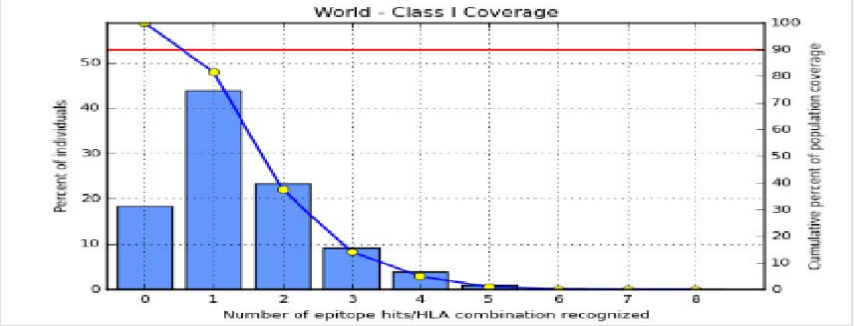

Figure 7: Selection of T-cell epitopes with the highest world population coverages shows EVLNRLTYNY has highest population coverages among all selected epitopes

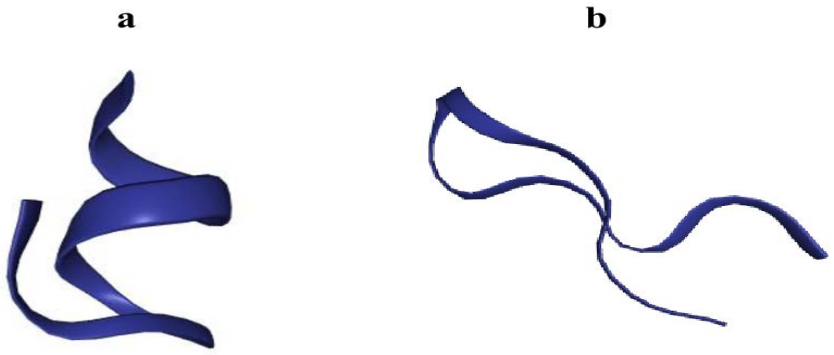

Figure 8: (a) Predicted 3D Structure of EVLNRLTYNY; (b) Predicted 3D Structure of FAYFTGELNI

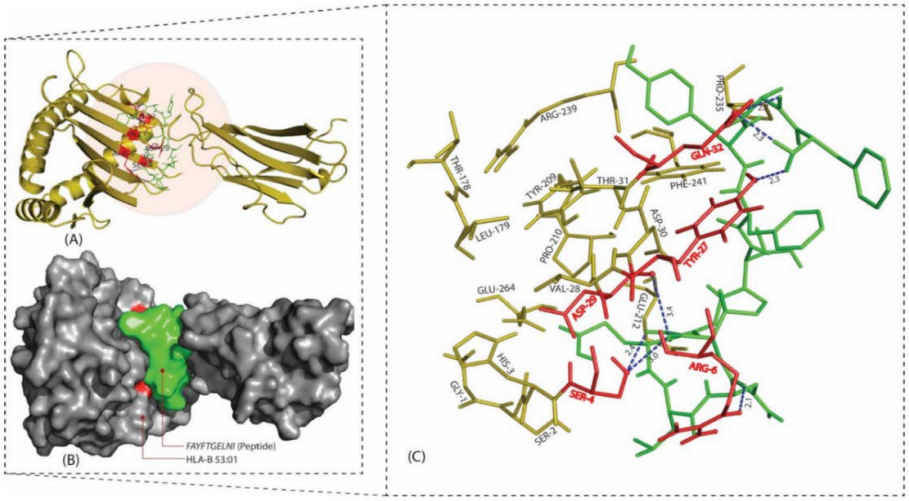

Figure 9A: (A & B) Peptide FAYFTGELNI S binds in HLA-B*53:01 groove; (C) Residues *SER-1, ARG-6, TYR-27, ASP-29* and *GLN-32* (Red colour) form regular hydrogen bonds while residues in oily yellow colour are surrounding residues

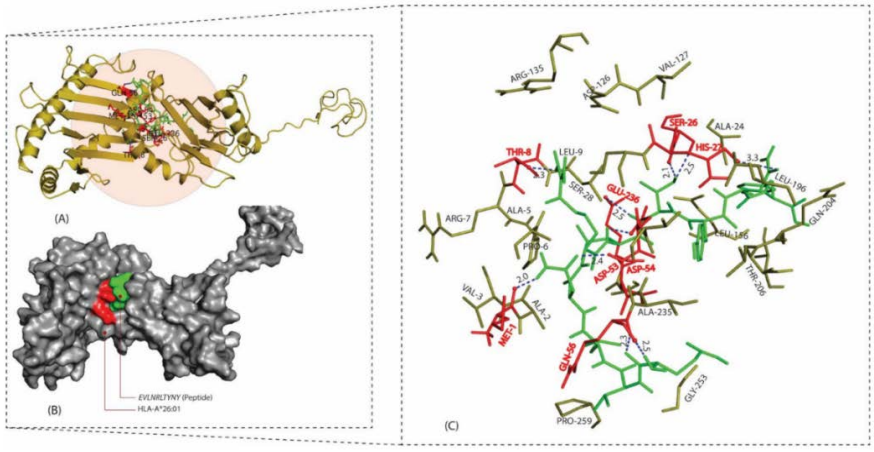

Figure 9B: Molecular docking results:(A & B) Lowest energy poses in HLA-A*26:01binding site for EVLNRLTYNY; (C) Residues *MET-1, THR-8, SER-26, HIS-27, ASP-53, ASP-54, GLN-56*and *GLU-236* (Red colour) form regular hydrogen bonds shown as blue dash lines with bond length while other residues (oily yellow colour) are surrounding residues

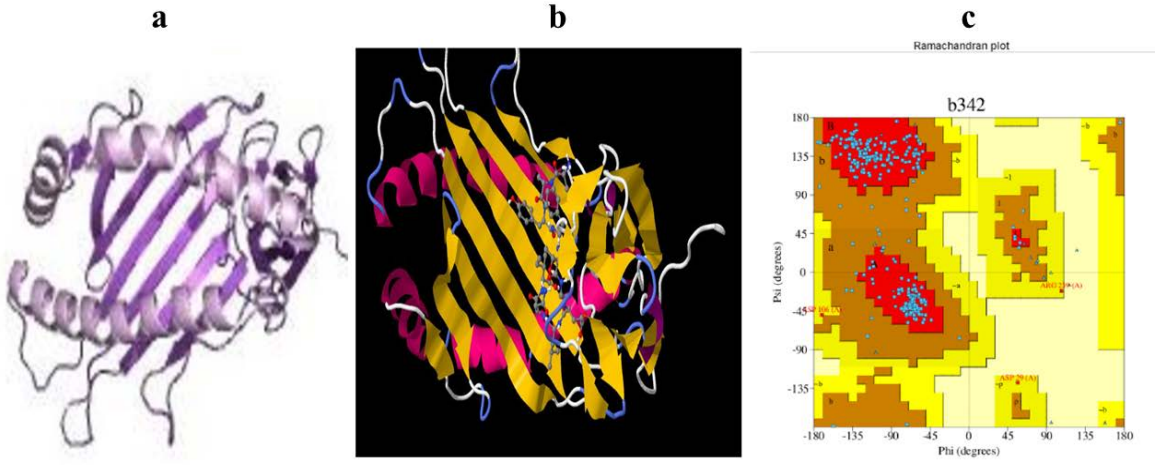

Figure 10: (a) Predicted 3D structure of the HLA-A*26:01 allele; (b) Molecular docking results of HLA-A*26:01 with EVLNRLTYNY: Binding energy vs RMSD plot shows the best conformation based on binding energy ranking or RMSD clustering; (c) Ramachandran plot of HLA-A*26:01 including stats depicting residues in its most favorable and disallowed regions and also the model's G-factor

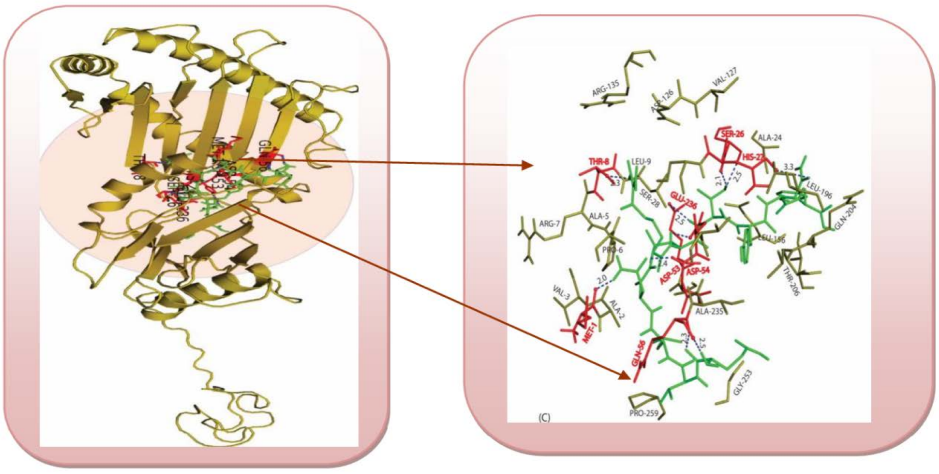

Figure 11: Represents the epitope EVLNRLTYNY which binds in the HLA-A*26:01 molecules groove

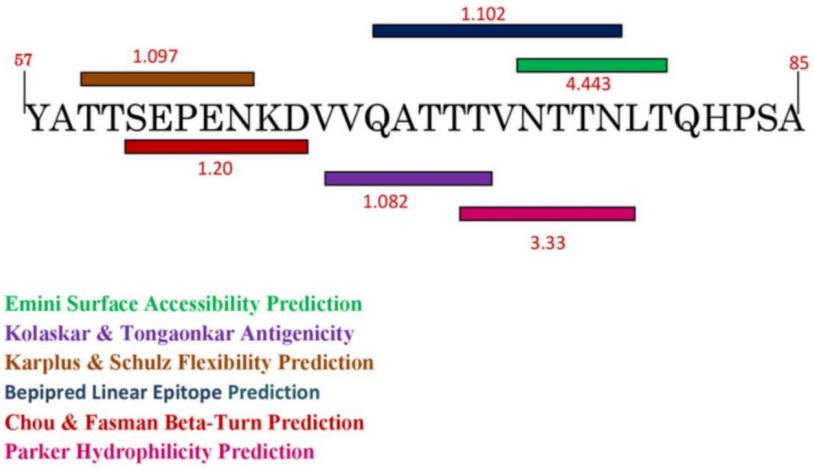

Figure 12: The combined B-cell linear epitope prediction

Newborns, with their still-developing immune systems and continual exposure to microorganisms, are at risk for a broad variety of potentially life-threatening acute illnesses.

Lower respiratory tract infection and sepsis are two of the top causes of death among babies less than 2 years old[67,68], the majority of whom are newborns[53,68]. The reported annual incidence of unexpected, unknown newborn fatality in the USA amounted to 100 deaths per 100,000 live births during 1999-2001[69]. When cross-checked these data, it provides further confirmation that parechoviruses can cause grave illness in babies. A total of 2,967 cases in the U.S. of HPeV diseases have been recorded in 2014-2016[66]. The global distribution shows that at a rather alarming rate, HPeVs is becoming widespread as a Nosocomial-associated febrile illness[70]. There is no commercial treatment like antiviral drugs and vaccines available for HPeVs yet[12]. The development of the HPeVs vaccine, therefore, has become rather vital[12,61]. A vaccine is made from an agent mimicking pathogenic microorganisms, generally prepared either from weakened or killed microbes or maybe consists of its surface proteins, toxins, etc[72]. Vaccines made from whole or part of organisms ' cells have several disadvantages. The safety issue with this type of vaccination is that it can lead to autoimmune reactions and severe allergic reactions.

The medium contaminants (antibiotics, eggs, etc.) used to grow microorganisms, not the disease, often triggered allergic reactions. Attenuated or inactivated pathogens may be unsuitable for vaccinations. Microbes can become pathogenic anytime. Subunit vaccinations, like whole pathogen vaccines, cause development problems and poor or unwanted immune responses[72]. Consequently, the safest and most sensitive vaccine development technique tends to be the use of minimal antigenic epitopes to generate the necessary immune response. Epitopes have become potential candidates for the vaccine, as it is considerably easier to build or generate, and there is no risk for infection and chemical stability. Besides protecting themselves from the disease, they act as therapeutic tools against the disease required to be treated. Epitopes are regions of antigens that are bound specifically by antibodies and are so readily identified by B and T cells in the immune system[73]. Even using a negligible microbe component that provides long-term protection against pathogens has become the phenomenon throughout the development of vaccines. Indeed, the future potential for the development of vaccines is fully synthetic peptide-based. This vaccine form could not replace the existing trend towards developing these recombinant protein-based vaccines soon. However, the development of peptide-based immunogens is also exciting[72].

It may take more than 10-15 years to develop a vaccine that could be tiring and complicated and often require public and private participation. Increasing knowledge of Sequence-based technology has helped in the advancements of genomics and proteomics of different viruses. The development of vaccines includes the selection of several antigenic epitopes that elicit an infection's immune response. We have conducted this study to look for novel and high-potential T-cell immunogenic epitopes. T-cell vaccines are more promising as they evoke prolonged immune responses and because the antigen can easily escape the antibody's memory response with antigenic drift. In designing peptide-based HPeV vaccines, the insilico approach was employed using vaccinomic and bioinformatic tools. Epitope vaccine prediction in silicon has already been used against diseases like Dengue, malaria, influenza, tumors, etc. The present study also aimed to detect possible B-cell epitopes in HPeV capsid polyprotein and MHC class I and MHC class II epitopes. A vaccine candidate epitope must fulfill many criteria, and based on all these criteria; we selected the seven most potent peptides. The first and foremost criterion is the Conservancy of the epitopes. The IEDB conservancy analysis tool was used to measure Conservancy. All the selected epitopes were found to have maximum (100%) identity. Out of the seven peptides, only EVLNRLTYNY and FAYFTGELNI were recognized to interact with the maximum number of alleles for MHC class I, but for both the peptides, the highest probable score was observed with HLA-A*26:01 alleles (Table 9; Figure 11).

However, the most prominent obstacle in vaccine development is allergenicity. Many vaccines are reportedly known to induce an allergic reaction by stimulating the immune system through Type 2 Helper cells and immunoglobulin E activation. The allergenicity scores for the two peptides proposed have been determined in this study to be 0.01 and were consequently regarded as non-allergic. The molecular docking studies are required to confirm that the target is bound to a specific region. Our findings from the epitope prediction workflow were validated, the epitope proposed for binding was confirmed, and the validity of the epitope in immune response induction was also predicted. The affinity of binding to the HLA antigen was ≤-6.10 kcal/mol, which is very promising and testifies, when applied in vivo, to the interaction between the cleft epitope and the HLA molecule. T-cells provide cell-mediated innate immunity by transferring information from the inflammatory site by recognizing pathogens using signatures (frequently referred to as epitopes). The prediction of CD4 + T cells epitope is thus an essential tool for vaccine development. We found 106 HLA-DRB 1 epitope with an IC50 < 100. We have found 106 HLA-

DRB 1 epitope with an IC50 < 100. We noticed that only two potential CD4+T cell epitopes (EVLNRLTYNY and FAYFTGELNI) interacted with most HLA DRB 1 molecules after the filtration criteria were applied. Residual amino acids in the 57-85 region inside the HPeV capsid Poly Protein were predicted to have B-cell epitopes (Figure 12). The predicted region is recognized as a B-cell epitope that induces the necessary immune response.

The vaccine is currently built generally based on the B-cells immunity. However, vaccines based on the T-cell epitope have been promoted as the host can produce a robust CD8+T cell immune response to the infected cell. We found that the predicted epitopes have been preserved throughout the evolution of all selected subtypes of viruses (presented in multiple sequences). Their results have shown a very high degree of resemblance[74]. Thus, if created, the predicted candidate epitope (peptide) will be considered a prospective broad-spectrum vaccine[74]. This experimental method of validation was recently achieved by insilico research[75], which identified in Mycobacterium tuberculosis a multi-epitopic cluster of secretory protein (Ag85B), linked to 15 class I and three class II molecules and subsequently validated in vitro[74].

Khan et al. (2014) selected MHCI and MHCII alleles with a higher affinity for the epitopes (IC50 < 500 nm), whereas we have chosen peptides with higher affinity (IC50 < 200 nm and IC50 < 100 nm). During our study, we followed the same *in silicon* strategy, but in some cases, the selection of MHC class I and class II molecules was found to be more specific. However, all such peptides with higher affinity (IC50 < 200 nm and IC50 < 100 nm) were chosen for MHC-I and MHC-II, respectively. Because each predicted T-cell epitope has established immunogenic properties by the value of its IC50, it implies the binding affinity between peptide and HLA-molecules and associated restricting HLA-alleles. Lower IC50 peptides were found well inhibited[76]. The peptides have also been predicted to be immunogenic and allergic. All predictions have been made in order to retain the high precision described by a specific threshold value. It has also been noted that the B-cell epitope could effectively direct the mapping of experimental epitopes so that the results of studies focused on anticorporeal affinities such as Western blotting, ELISA, and the radioimmune assay can be interpreted. We suggested that the epitopes proposed could induce an efficient immune response like *in vivo* peptide vaccines.

CONCLUSION

An epitope-based universal peptide vaccination against all pathogenic strains of Human parechoviruses (HPeVs) is a goal of this study, and possible epitopes are identified. B-cell epitopes are predicted to be QATTTVNTTN, whereas T-cell epitopes are predicted to be EVLNRLTYNY.

Authorship Contributions

Shaia S R Almalki: Project administration, Supervision, Conceptualization, Formal analysis, Writing – review and editing of the original draft

Shazia Shaheen Mir: Project administration, Investigation, Resources, Experimental design and methodology, Formal analysis, Writing – review and editing of the original draft

Abdulmajeed A A Sindi: Resources, Characterization, Data curation, Formal analysis

Mohammad O Alzahrani: Resources, Characterization, Data curation, Formal analysis

Naseem Akhter: Conceptualization, Formal analysis, Validation, Visualization, Writing – review and editing of the original draft

Read A Alharbi: Investigation, Resources, Formal analysis, Validation, Visualization

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Potential Conflict of Interest: None

Competing Interest: None

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