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Modeling and in Silico Analysis for Prediction of Epitopes Vaccine against Norwalk virus from Capsid Protein (VP1) through Reverse Vaccinology

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Abstract Noroviruses are the leading cause of acute gastroenteritis and is responsible for approximately 685 million cases and 200,000 deaths annually worldwide. Currently, there is no vaccine to prevent human norovirus infection, and there is no specific therapy available to treat it. This study aimed to predict epitopes from the capsid VP1 protein that elicited the immune system and acted as safer efficacious vaccine. A total of 21 noroviurse strains were retrieved from the NCBI database. The IEDB analysis resources were used for epitopes prediction against B and T cells. The population coverage was calculated for the proposed epitopes against the whole world. Eight epitopes ($_{48}$QVNP$_{51}$, $_{159}$EVPLE$_{163}$, $_{224}$VEQK$_{227}$, $_{245}$RAPLP$_{249}$, $_{376}$ISPPS$_{380}$, $_{409}$VYPP$_{412}$, $_{473}$FKAY$_{476}$ and $_{492}$PQQLP$_{496}$) successfully passed all B cell prediction tools and were shown to be antigenic, nonallergic and nontoxic. Thus were proposed as B cells epitopes. For cytotoxic T cells, a total of 103 epitopes were found to interact with MHC-I alleles. However, only 22 epitopes were shown to be antigenic, nonallergic and nontoxic. Among them four epitopes namely ($_{140}$-AQATLFPHV-$_{148}$; $_{216}$-FLFLVPPTV-$_{224}$; $_{499}$-GVFVFVSWV-$_{507}$ and $_{410}$-YPPGFGEVL-$_{418}$) interacted with high number of MHC-I alleles and demonstrated favourable population coverage and thus were proposed as cytotoxic T lymphocytes MHC-1 epitopes. Moreover helper T cells, a total of 421 core epitopes were found to interact with MHC-II alleles. However, only 105 epitopes were shown to be antigenic, nonallergic and nontoxic. Eight epitopes namely ($_{216}$-FLFLVPPTV-$_{224}$; $_{499}$-GVFVFVSWV-$_{507}$; $_{433}$-LPCLLPQEY-$_{441}$; $_{90}$-NPFLLHLSQ-$_{98}$; $_{394}$-NYGSSITEA-$_{402}$; $_{247}$-PLPISSMGI-$_{255}$; $_{220}$-VPPTVEQKT-$_{228}$; $_{410}$-YPPGFGEVL-$_{418}$) were interacted with most frequent MHC class II alleles, demonstrated higher population coverage and three of them ($_{216}$-FLFLVPPTV-224; $_{499}$-GVFVFVSWV-$_{507}$ and $_{410}$-YPPGFGEVL-$_{418}$) were shown to interact with both MHC-I and MHC-II alleles. Therefore they were proposed as T helper cells epitopes. The population coverage was 60.35% and 99.96% for MHC-I and MHC-II epitopes, respectively, and 100% for all T cells epitopes. Taken together 17 epitopes successfully proposed as vaccine candidate against noroviruse. *In vivo* and *in vitro* clinical trials studies are required to elucidate the effectiveness of these epitopes as vaccine.

Keywords: *Noroviruses, NCBI, IEDB, Insilico vaccine, B cells, T cells*

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1. Introduction

Noroviruses are the leading cause of acute gastroenteritis and is responsible for approximately 685 million cases and 200,000 deaths annually worldwide [1,2]. These viruses cause gastrointestinal disease, resulting in recurrent vomiting and diarrhea [1,3,4]. The infection mostly characterized by high excreted viral loads [5], severe infectivity [6] and short-term immunity [7].

Norwalk viruses are the main cause of outbreak and irregular of nonbacterial gastroenteritis [8,9] and connected to almost one over five of all reported cases are acute gastroenteritis internationally [9,10]. Contamination causes outbreaks of water - borne and food - borne illness in the society and generates signs of vomiting and diarrhea [11-14]. Diarrheal diseases are associated with high mortality rates [15-19] and noroviruses are an important cause of epidemic gastroenteritis together in children and adults worldwide [10,20-24].

Noroviruses are an assemblage of single, positive-stranded RNA viruses classified into the Norovirus genus in the family Caliciviridae [11,25,26,27]. This family composed of five types; Norovirus, Sapovirus, Lagovirus, Vesivirus and Nebo virus. The first two genera are human while the

others are animal genera [11,12,17,18,27]. Genetically the human noroviruses are dissimilar [19] and are divided into six genogroups based on the amino acid sequence of the major structural proteins VP1 and VP2 [10,11,12,18].

The norovirus genome has three open reading frames (ORFs) of which ORF2 and ORF3 encode the major capsid protein (VP1) that determines the antigenicity of the virus, as well as the minor capsid protein (VP2). ORF1 encodes a large polyprotein that is cleaved by the viral protease in mature non-structural proteins, including the RNA-dependent RNA polymerase [28]. To date, all norovirus vaccine candidates contain non-infectious recombinant VP1 proteins, either as virus-like particles (VLP), as P-particles, or as recombinant adenoviruses [29].

No vaccine is obtainable to put off norovirus illness or infection [8,21], however there was attempts to make a vaccine from a novel strain of NOVs as plant - based oral vaccine, but unfortunately was invaluable [30]. In this study we attempted to propose an epitopes from the capsid protein (VP1) of the virus that could elicited both B and T lymphocytes and act as safer vaccine.

2. Materials and Methods

2.1. Protein Sequence Retrieval and Alignment Tool

The protein sequences of 21 capsid proteins (VP1) were retrieved from the NCBI database (https: //www.ncbi.nlm.nih.gov/). The retrieved strains, accession numbers, country and date of collection were shown in (Table 1).

Table 1. Retrieved strains of capsid protein (VP1) with their date of collection, accession numbers and geographical regions

Country	Accession number	Year
USA	NP_056821.2	2000
USA	AGT17792.1	1968
USA	AGM33244.1	2009
USA	AGM33241.1	2010
USA	AGM33238.1	2008
USA	AGM33235.1	2009
USA	AGM33232.1	2011
USA	AGM33229.1	2009
USA	AGM33226.1	2009
USA	AGM33223.1	2011
USA	AGM33220.1	2010
USA	AGM33217.1	2008
USA	AGM33214.1	2009
USA	AGM33211.1	2009
USA	AGM33208.1	2010
USA	AGT17836.1	1978
USA	AGT17826.1	1979
USA	AGT17809.1	1973
USA	AGT17806.1	1974
USA	AGT17803.1	1973
USA	AGT17785.1	1972

Determination of the conserved regions

The retrieved protein sequences were further aligned to obtain conserved regions using multiple sequence alignment (MSA) tools, Clustal W in the BioEdit program, version 7.0.9.0 [31]. Multiple sequence alignment (MSA) analysis was performed to analyze the conserved residue sequence amongst the screened B and T cell epitopes.

2.2. Evolution Analysis

The retrieved capsid protein sequences (VP1) were subjected to evolutionary divergence analysis and a phylogenetic tree was constructed to determine the common ancestor of each retrieved strain using MEGA7.0.26 (7170509) software [32].

2.3. Determination of B Cells Epitopes

The conserved regions of the candidate epitopes were analyzed by different prediction software tools obtained by Immune Epitope Database (IEDB) analysis (https: //www.iedb.org/). The reference sequence of VP1 was used as an input for the IEDB software resource analysis.

2.4. B Lymphocytes Epitopes Prediction

Tools from IEDB were used to identify the B cell epitopes (http: //tools.iedb.org/bcell/). This includes Bepipred linear epitope prediction analysis [33], Emini surface accessibility prediction [34] and Kolaskar and Tongaonkar antigenicity scale [35]. These methods predicted specific epitopes in the capsid protein that were linear, at surface and immunogenic, respectively, and can bind to B cell receptors

2.5. T-lymphocytes Epitopes Prediction

The IEDB tools were used for the identification of the T cell epitopes prediction. The prediction method includes the major histocompatibility complex class I and Π (MHC-I, MHC-Π)

2.6. MHC-I Binding Predictions

Analysis of epitopes binding to MHC-I molecules was assessed by the software in IEDB MHC-I prediction tools (http: //tools.iedb.org/mhci/). The prediction method was obtained by Artificial Neural Network (ANN), Stabilized Matrix Method (SMM) or Scoring Matrices derived from combinatorial peptide libraries. Before the prediction step, epitopes lengths were set as 9mers. The conserved epitopes that bind to alleles at score equal to or less than 300 was considered as half-maximal inhibitory concentrations. Epitopes equal to or less than the IC50 were selected for further analysis

2.7. MHC-Π Binding Predictions

Analysis of epitopes binding to MHC-II molecules was performed by the IEDB MHC-II prediction tools (http: //tools.iedb.org/mhcii/). The neural networks align (NN-align) that allow for simultaneous identification of the MHC-II binding core epitopes and binding affinity was used. All the predicted conserved epitopes that bind to many alleles at score equal to or less than 3000 half-maximal inhibitory concentration (IC50) was

considered. Epitopes that equal to or less than the IC50 were selected for further analysis.

2.8. Antigenicity of the Predicted Epitopes

For determination of the antigenicity of the predicted epitopes, the sequence of the predicted epitopes for B and T lymphocytes were submitted to the VaxiJen v2.0 server (http: //www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) for antigenicity prediction. The threshold of VaxiJen v2.0 server was set to the default threshold (0.4). Epitopes with antigenicity were further investigated for allergenicity and toxicity.

2.9. Allergenicity and Toxicity of the Predicted Epitopes

The following servers; AllergenFP [36], AllerTOP [37], Allermatch [38] and PA^{3}P [39] were used to predict the allergenicity of the proposed epitopes for B and T lymphocytes. Epitopes found to be non-allergenic were further assessed for toxicity level by ToxinPred server [40].

2.10. Population Coverage Analysis of Predicted Epitopes

For the calculation of the population coverage for all potential MHC-I and II epitopes, the IEDB tools was used (http: //tools.iedb.org/population/). The capsid protein was assessed for population coverage against the whole world with selected MHC-I and MHC-II interacted alleles.

2.11. Homology Modeling

Raptor X for 3D structure prediction server (http: //raptorx.uchicago.edu/StructurePrediction/predict/) was used for creation the 3D structure of the reference protein. The reference sequence was used as an input and Chimera 1.8 [41] was used as a tool to visualize the selected epitopes belonging to B cell and T cell (MHC-I and MHC-II). Homology modeling was used for visualization of the surface accessibility of the B lymphocytes predicted candidate epitopes as well as for visualization of all predicted T cell epitopes in the structural level.

3. Results

3.1. Phylogenetic Tree

According to the result of the conserved regions of the retrieved strains of the VP1 proteins using multiple sequence alignment analysis, the protein sequences demonstrated various strains with less mutated regions. This resulted in closed relationship of the retrieved sequences despite the possibilities of mutations in VP1 capsid protein. Generally Figure 1 provided the phylogenetic tree of the retrieved strains and the strains demonstrated molecular evolutionary divergence.

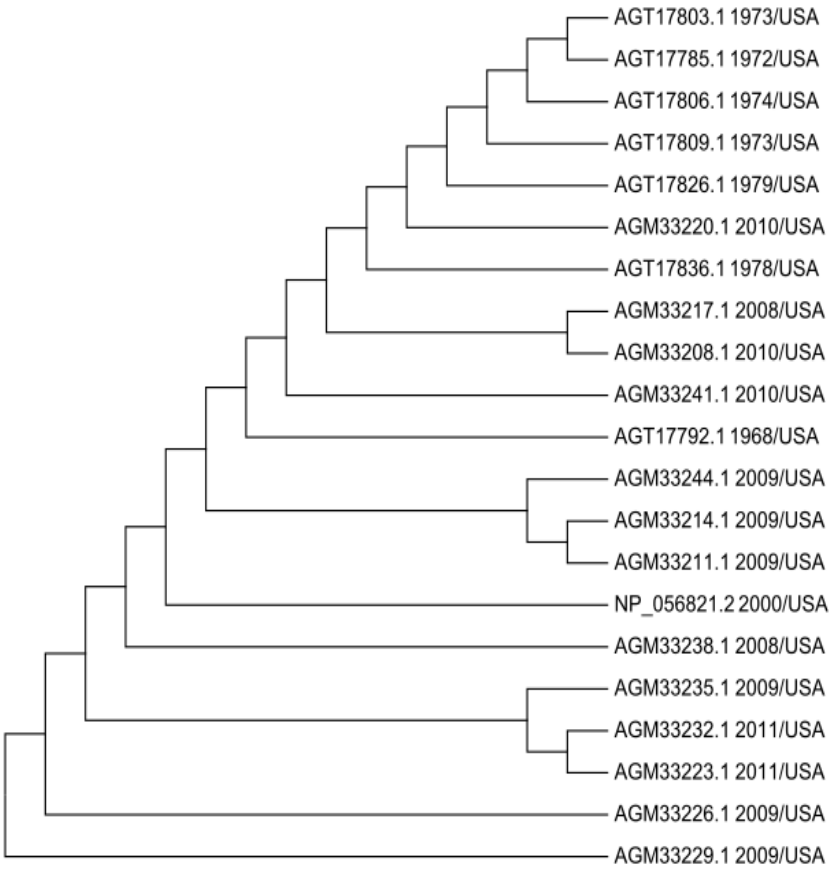

Figure 1. Phylogenetic tree of the capsid protein (VP1) of the retrieved strains. The retrieved strains demonstrated divergence in their common ancestors

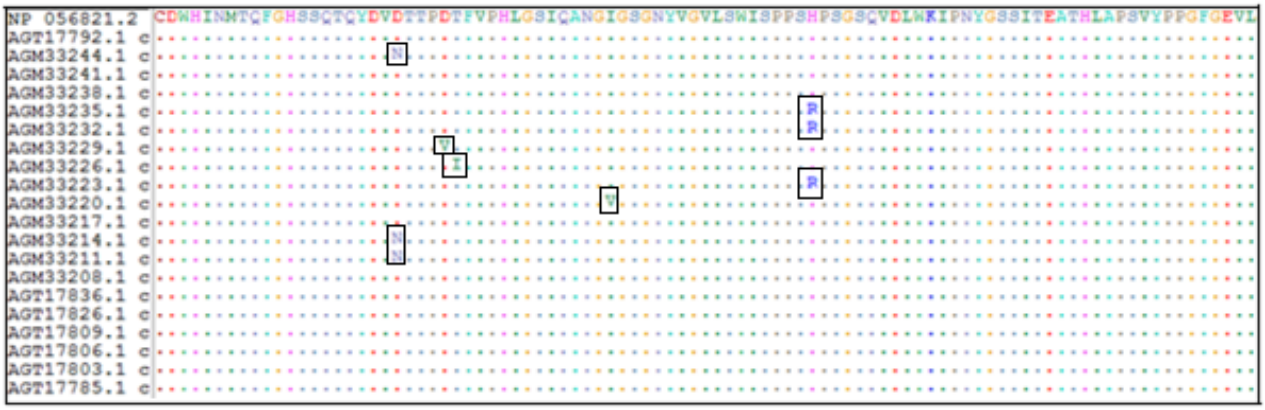

Figure 2. Multiple sequence alignment (MSA) of the retrieved strains of the capsid protein (VP1) using Bioedit software and ClustalW. Dots indicated the conservancy of the retrieved strains and letters within the rectangular indicated no conservancy (mutation) in amino acid

3.2. Alignment

The multiple sequence alignment was performed to obtain the regions of conservancy among Norwalk virus variants using multiple sequence alignment (MSA) tools, of Clustal W in the BioEdit program, version 7.0.9.0. As shown in Figure 2 the dots meant that amino acids at that position were conserved while the letters meant that the amino acids at that position were mutated. Generally the retrieved strains demonstrated less mutated regions during the alignment process. Thus multiple epitopes were predicted to be conserved among the retrieved strains. It is noteworthy that only the conserved epitopes (100% conserved epitopes) were selected for further analysis. Thus the proposed epitopes elected when they showed 100% conservancy among the retrieved strains

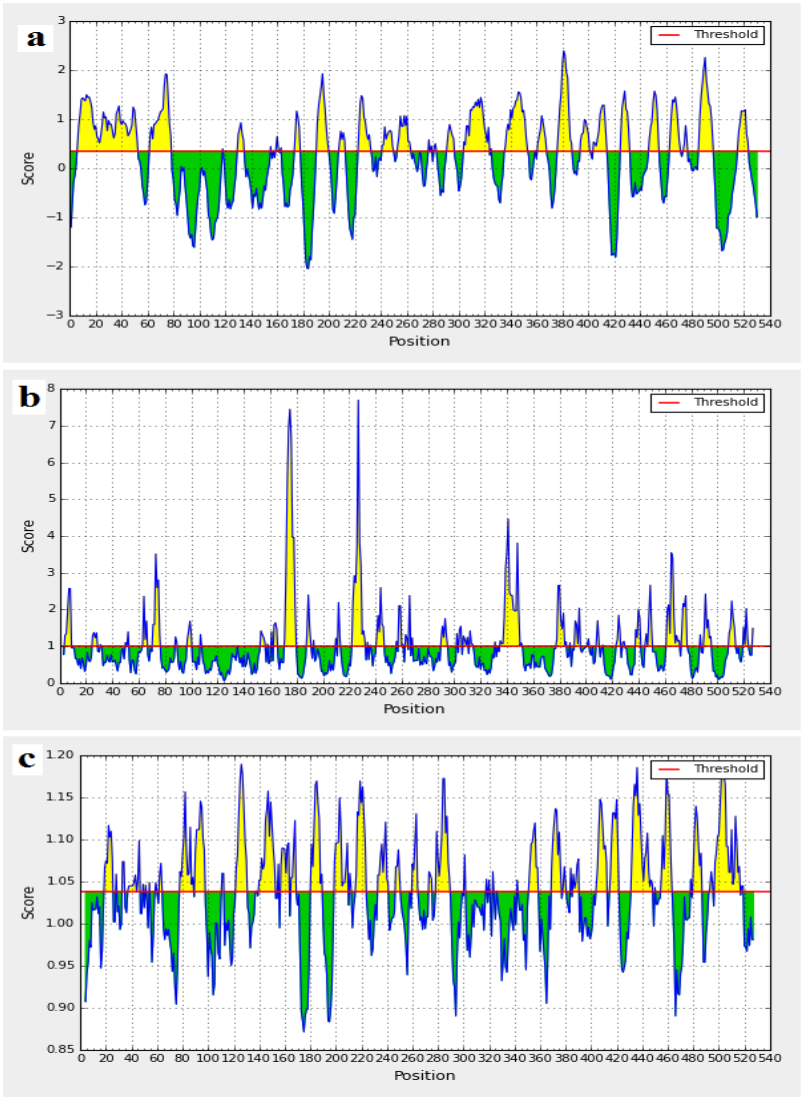

Figure 3. Prediction of B-cell epitopes by different IEDB scales (**a-** Bepipred linear epitope prediction, **b-** Emini surface accessibility, **c-** Kolaskar and Tongaonkar antigenicity prediction) for the capsid protein. Regions above threshold (red line) were proposed as a part of B cell epitope while regions below the threshold (red line) were not

Table 2. B-cell epitopes prediction from the capsid protein, the position of peptides is according to the position of amino acids in the capsid protein

Linear peptides	Start	End	Length	Emini 1.000	Kolaskar 1.038
KDATSSVDGASGAGQLVPEVNASDPLAMDPVAGSSTAVAT	6	45	40	0.621	1.032
QLVPE*#	20	24	5	1.025	1.113
GQVNPI	47	52	6	0.651	1.044
QVNP*#	48	51	4	1.417	1.059
QAPQGEFTISPNNTPGD	62	78	17	5.175	0.963
QAPQ*#	62	65	4	2.077	1.039
PPGFGS	129	134	6	0.798	0.996
EVPLE#	159	163	5	1.025	1.08
DRNQ	174	177	4	4.039	0.882
RTGGGTGDSF	191	200	10	1.014	0.916
TCPSP	208	212	5	0.895	1.092
TVEQKTRPFT	223	232	10	4.695	0.993
VEQK*#	224	227	4	1.974	1.045
LSNSRAPLPI	241	250	10	0.872	1.052
RAPLP*#	245	249	5	1.408	1.063
MGISPDNVQSV	253	263	11	0.541	1.033
DNVQSV*#	258	263	6	1.009	1.073
RGTSNG	291	296	6	1.752	0.886
LDGTPFHPFEGPAPIGFPDLGG	304	325	22	0.379	1.013
PFHP*#	308	311	4	1.249	1.081
ISPPS#	376	380	5	1.086	1.061
SPPS*#	377	380	4	1.904	1.038
PSGSQV#	382	387	6	1.038	1.06
NYGSSITEA	394	402	9	1.286	0.979
HLAPSVYPPGF	404	414	11	0.62	1.103
VYPP*#	409	412	4	1.233	1.168
MPGPGAYN	425	432	8	1.163	0.963
SEQAPTVG	447	454	8	1.312	1.022
QAPTV*#	449	453	5	1.046	1.087
DPDTGRNLG	463	471	9	2.568	0.928
FKAY#	473	476	4	1.215	1.062
PNGASSGPQQLP	485	496	12	2.414	1.007
PQQLP*#	492	496	5	2.135	1.082
PVGTASSAR	515	523	9	0.955	1.028

*peptides revealed higher score if they were shorten in all tools.
#Epitopes that passed all the B cell prediction methods and were further subjected to antigenicity, allergenicity and toxicity investigation.

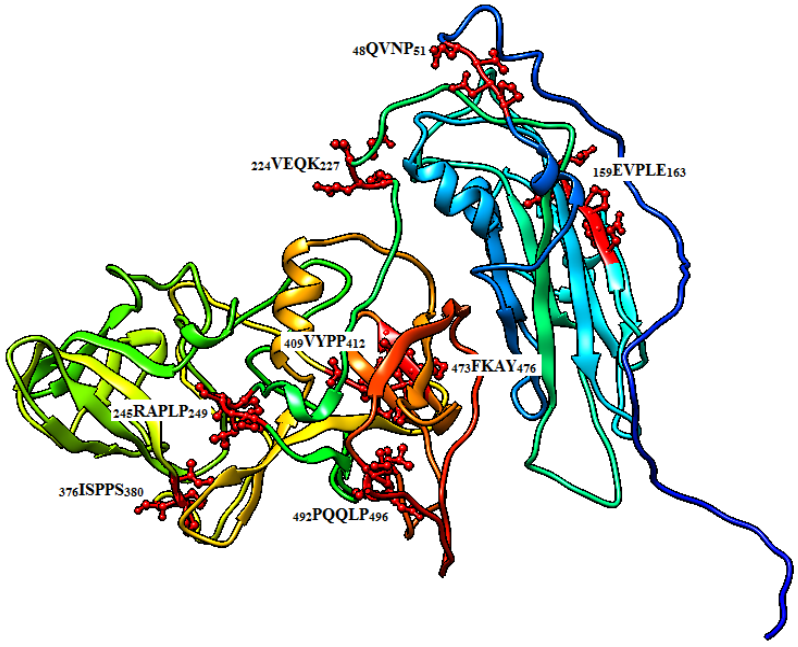

Figure 4. Position of proposed eight conserved B cell epitopes in structural level of the capsid protein (VP1). The epitopes were shown in dark red ball and sticks shapes. These epitopes showed conservancy, high score in surface accessibility and antigenicity using IEDB software, nonallergic and nontoxic. The position of these epitopes was according to their position in the capsid protein

Table 3. The 15 epitopes of the B-cell that overlapped the Bepipred linear epitope prediction, Emini surface accessibility and Kolaskar and Tongaonkar antigenicity prediction tools and further subjected to antigenicity, allergenicity and toxicity. The position of epitopes is according to the position of amino acids in the capsid protein (VP1)

Peptide	Start	End	Length	Antigenicity	Allergenicity	Toxicity
QLVPE	20	24	5	0.4	Allergen	Non-Toxin
QVNP#	48	51	4	0.4	non-allergen	Non-Toxin
QAPQ	62	65	4	0.4	Allergen	Non-Toxin
EVPLE#	159	163	5	0.4	non-allergen	Non-Toxin
VEQK#	224	227	4	0.4	non-allergen	Non-Toxin
RAPLP#	245	249	5	0.4	non-allergen	Non-Toxin
DNVQSV	258	263	6	0.7901	Allergen	Non-Toxin
PFHP	308	311	4	0.4	Allergen	Non-Toxin
ISPPS#	376	380	5	0.4	non-allergen	Non-Toxin
SPPS	377	380	4	0.4	Allergen	Non-Toxin
PSGSQV	382	387	6	0.632	Allergen	Non-Toxin
VYPP#	409	412	4	0.4	non-allergen	Non-Toxin
QAPTV	449	453	5	0.4	Allergen	Non-Toxin
FKAY#	473	476	4	0.4	non-allergen	Non-Toxin
PQQLP#	492	496	5	0.4	non-allergen	Non-Toxin

#The proposed B cell epitopes.

3.3. Prediction of B Cell Epitope

The capsid protein (VP1) sequence was subjected to Bepipred linear epitope, Emini surface accessibility and Kolaskar and Tongaonkar antigenicity methods in IEDB. Figure 3 demonstrated the thresholds of the methods used to predict the B cell epitopes. For instance the Bepipred linear epitopes prediction method showed a threshold binding score to B cell of 0.350 (minimum -0.005 and maximum 2.401). This method predicted thirty four linear epitopes eliciting the B cell from the conserved regions. In Emini surface accessibility the prediction threshold of the surface accessibility area of the epitopes was 1.000 (minimum of 0.054 and maximum of 7.701). Twenty five epitopes were potentially in the surface by passing the default threshold 1.000. For Kolaskar and Tongaonkar antigenicity the average of antigenicity was 1.038 (minimum of 0.871 and maximum of 1.190). Nineteen epitopes gave score above the default threshold 1.038. All these epitopes and their scores against the B cell were provided in Table 2. However, only fifteen epitopes successfully overlapped the three tools and were shown in Table 3. These fifteen epitopes were further investigated for their antigenicity, allergenicity and toxicity. Eight epitopes namely ($_{48}$QVNP$_{51}$, $_{159}$EVPLE$_{163}$, $_{224}$VEQK$_{227}$, $_{245}$RAPLP$_{249}$, $_{376}$ISPPS$_{380}$, $_{409}$VYPP$_{412}$, $_{473}$FKAY$_{476}$ and $_{492}$PQQLP$_{496}$) were shown to be antigenic, nonallergic and nontoxic and thus were proposed as B cell epitopes. The positions of the proposed epitopes in the 3D structural level of VP1 capsid protein were shown in Figure 4.

Table 4. The 22 epitopes that interacted with MHC-1 from the capsid protein (VP1) of the Norwalk virus that demonstrated antigenicity, nonallergic and nontoxic. The population coverage for each predicted epitope was calculated and election of the proposed epitopes was based on the higher population coverage score. The position of epitopes is according to the position of amino acids in the capsid protein

Peptide	Start	End	Antigenicity	Threshold	Allergenicity	Toxicity	PC%
APTVGEAAL	450	458	ANTIGEN	0.5389	NON-ALLERGEN	Non-Toxin	15.92
AQATLFPHV#	140	148	ANTIGEN	0.464	NON-ALLERGEN	Non-Toxin	45.62
ARGRLGLRR	521	529	ANTIGEN	0.7466	NON-ALLERGEN	Non-Toxin	4.78
DPVAGSSTA	34	42	ANTIGEN	0.7453	NON-ALLERGEN	Non-Toxin	8.42
EATHLAPSV	401	409	ANTIGEN	1.2979	NON-ALLERGEN	Non-Toxin	2.5
EQKTRPFTL	225	233	ANTIGEN	1.1033	NON-ALLERGEN	Non-Toxin	10.55
FLFLVPPTV#	216	224	ANTIGEN	0.5167	NON-ALLERGEN	Non-Toxin	46.73
GVFVFVSWV#	499	507	ANTIGEN	0.5765	NON-ALLERGEN	Non-Toxin	40.6
KIRGTSNGT	289	297	ANTIGEN	0.7144	NON-ALLERGEN	Non-Toxin	3.89
KTRPFTLPN	227	235	ANTIGEN	0.6615	NON-ALLERGEN	Non-Toxin	3.89
LPCLLPQEY	433	441	ANTIGEN	0.496	NON-ALLERGEN	Non-Toxin	8.42
MPGPGAYNL	425	433	ANTIGEN	0.835	NON-ALLERGEN	Non-Toxin	23.07
NMRVRIMLA	106	114	ANTIGEN	0.4337	NON-ALLERGEN	Non-Toxin	14.03
NYVGVLSWI	368	376	ANTIGEN	0.7341	NON-ALLERGEN	Non-Toxin	5.43
PEVNASDPL	23	31	ANTIGEN	0.9599	NON-ALLERGEN	Non-Toxin	7.81
QSVQFQNGR	261	269	ANTIGEN	0.936	NON-ALLERGEN	Non-Toxin	11.03
RAPLPISSM	245	253	ANTIGEN	0.5936	NON-ALLERGEN	Non-Toxin	20.74
SPNNTPGDV	71	79	ANTIGEN	0.5505	NON-ALLERGEN	Non-Toxin	12.87
VYPPGFGEV	409	417	ANTIGEN	0.4048	NON-ALLERGEN	Non-Toxin	3.04
YNGWVGNMR	100	108	ANTIGEN	1.22	NON-ALLERGEN	Non-Toxin	5.83
YPPGFGEVL#	410	418	ANTIGEN	0.4272	NON-ALLERGEN	Non-Toxin	26.51
YQLKPVGTA	511	519	ANTIGEN	1.6335	NON-ALLERGEN	Non-Toxin	1.95

PC: population coverage
#the proposed epitopes.

Table 5. The best four proposed epitopes from the capsid protein (VP1) of the Norwalk virus that interacted with MHC class I alleles. The position of epitopes is according to the position of amino acids in the capsid protein

Peptide	Start	End	Allele	ic50	Percentile Rank
AQATLFPHV	140	148	HLA-A*02: 01	49.32	0.53
			HLA-A*02: 06	2.99	0.02
			HLA-B*15: 01	201.61	0.86
FLFLVPPTV	216	224	HLA-A*02: 01	3.65	0.02
			HLA-A*02: 06	41.52	0.47
			HLA-C*12: 03	39.62	0.1
GVFVFVSWV	499	507	HLA-A*02: 01	147.42	1.4
			HLA-A*02: 06	117.08	1.2
YPPGFGEVL	410	418	HLA-B*07: 02	143.39	0.52
			HLA-B*39: 01	198.65	0.18
			HLA-C*12: 03	106.34	0.2
			HLA-C*14: 02	259.86	0.36

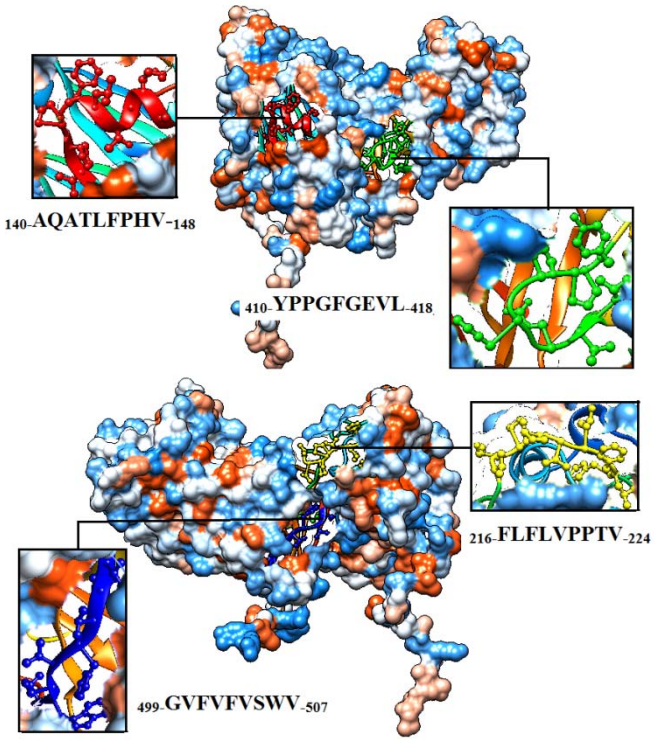

Figure 5. T cell proposed epitopes that interact with MHC-I alleles. Four epitopes ($_{140}$-AQATLFPHV-$_{148}$; $_{216}$-FLFLVPPTV-$_{224}$; $_{499}$-GVFVFVSWV-$_{507}$ and $_{410}$-YPPGFGEVL-$_{418}$) were shown in this figure since they interacted with MHC-1 alleles. The positions of these proposed epitopes was according to their position in the capsid protein

3.4. Prediction of Cytotoxic T-lymphocyte Epitopes and Interaction with MHC Class I

The reference sequence of the capsid protein was analyzed using IEDB MHC-1 binding prediction tools to predict T cell epitopes interacting with MHC Class I alleles. Based on Artificial Neural Network (ANN) with half-maximal inhibitory concentration (IC50)≤300, 103 epitopes were predicted to interact with different MHC-1 alleles. All these epitopes were further investigated for their antigenicity, allergenicity and toxicity. Only 22 epitopes were shown to be antigenic, nonallergic and nontoxic. Furthermore these 22 epitopes were investigated for their population coverage against the whole world. The 22 epitopes and their population coverage were shown in Table 4. Four epitopes namely $_{140}$-AQATLFPHV-$_{148}$; $_{216}$-FLFLVPPTV-$_{224}$; $_{499}$-GVFVFVSWV-$_{507}$ and $_{410}$-YPPGFGEVL-$_{418}$ demonstrated favourable population coverage and thus were proposed as cytotoxic T lymphocytes MHC-1 epitopes. The proposed four epitopes with their corresponding MHC-1 alleles were shown in Table 5. The positions of the proposed epitopes in the 3D structural level of capsid protein were shown in Figure 5.

3.5. Prediction of T Helper Cell Epitopes and Their Interaction with MHC Class II

The reference sequence of VP1 capsid protein was analyzed using IEDB MHC-II binding prediction tools. Based on NN-align with half-maximal inhibitory concentration IC50≤3000 there were 421 predicted epitopes found to interact with MHC-II alleles. When these epitopes subjected to antigenicity, allergenicity and toxicity investigations, only 105 epitopes were shown to be antigenic, non-allergic and nontoxic and the population coverage of each epitope alleles was calculated. The 105 epitopes were provided in Table 6.

Table 6. The 105 epitopes from the capsid protein (VP1) and their interaction with MHC class II. These epitopes demonstrated antigenicity, nonallergic and nontoxic. The population coverage for each predicted epitope was calculated and election of the proposed epitopes was based on the higher population coverage score.

Epitope	Antigenicity	Threshold	Allergenicity	toxicity	PC%
AALLHYVDP	ANTIGENIC	0.7926	NON-ALLERGEN	Non-Toxin	4.77
AGQLVPEVN	ANTIGENIC	0.6773	NON-ALLERGEN	Non-Toxin	56.45
AGQVNPIDP	ANTIGENIC	1.3491	NON-ALLERGEN	Non-Toxin	4.77
APIGFPDLG	ANTIGENIC	1.6097	NON-ALLERGEN	Non-Toxin	72.79
ARGRLGLRR	ANTIGENIC	0.7466	NON-ALLERGEN	Non-Toxin	0
ASDPLAMDP	ANTIGENIC	0.5582	NON-ALLERGEN	Non-Toxin	52.05
ATAGQVNPI	ANTIGENIC	0.9878	NON-ALLERGEN	Non-Toxin	80.15
AVATAGQVN	ANTIGENIC	0.703	NON-ALLERGEN	Non-Toxin	51.09
AYNLPCLLP	ANTIGENIC	1.2262	NON-ALLERGEN	Non-Toxin	61.65
DNVQSVQFQ	ANTIGENIC	1.1295	NON-ALLERGEN	Non-Toxin	17.84
DVDTTPDTF	ANTIGENIC	0.4424	NON-ALLERGEN	Non-Toxin	95.64
DWHINMTQF	ANTIGENIC	1.433	NON-ALLERGEN	Non-Toxin	73.42
EAALLHYVD	ANTIGENIC	0.7284	NON-ALLERGEN	Non-Toxin	95.01
EATHLAPSV	ANTIGENIC	1.2979	NON-ALLERGEN	Non-Toxin	41.45
EGPAPIGFP	ANTIGENIC	0.7762	NON-ALLERGEN	Non-Toxin	73.51
ELDGTPFHP	ANTIGENIC	0.925	NON-ALLERGEN	Non-Toxin	64.52
EQKTRPFTL	ANTIGENIC	1.1033	NON-ALLERGEN	Non-Toxin	10.54
EVNASDPLA	ANTIGENIC	0.5974	NON-ALLERGEN	Non-Toxin	71.49
FGSHNLTIA	ANTIGENIC	0.9286	NON-ALLERGEN	Non-Toxin	94.39
FHNNDRNQQ	ANTIGENIC	0.6906	NON-ALLERGEN	Non-Toxin	83.57
FLFLVPPTV#	ANTIGENIC	0.5167	NON-ALLERGEN	Non-Toxin	88.87
FYQLKPVGT	ANTIGENIC	1.058	NON-ALLERGEN	Non-Toxin	75.47
GDVLFDLSL	ANTIGENIC	0.8993	NON-ALLERGEN	Non-Toxin	83.15
GEFTISPNN	ANTIGENIC	1.249	NON-ALLERGEN	Non-Toxin	11.53
GFGSHNLTI	ANTIGENIC	1.1128	NON-ALLERGEN	Non-Toxin	18.23
GHSSQTQYD	ANTIGENIC	0.6946	NON-ALLERGEN	Non-Toxin	74.69
GIGSGNYVG	ANTIGENIC	0.9043	NON-ALLERGEN	Non-Toxin	49.64
GNYVGVLSW	ANTIGENIC	0.6499	NON-ALLERGEN	Non-Toxin	34.55
GQLVPEVNA	ANTIGENIC	0.7099	NON-ALLERGEN	Non-Toxin	85.75
GSHNLTIAQ	ANTIGENIC	0.5411	NON-ALLERGEN	Non-Toxin	54.85
GVFVFVSWV#	ANTIGENIC	0.7341	NON-ALLERGEN	Non-Toxin	79.44
HLAPSVYPP	ANTIGENIC	0.8546	NON-ALLERGEN	Non-Toxin	91.31
IADVRTLDP	ANTIGENIC	1.3707	NON-ALLERGEN	Non-Toxin	58.94
IEVPLEDVR	ANTIGENIC	0.7191	NON-ALLERGEN	Non-Toxin	4.77
INGVFVFVS	ANTIGENIC	0.5174	NON-ALLERGEN	Non-Toxin	77.12
ISHLASEQA	ANTIGENIC	0.4341	NON-ALLERGEN	Non-Toxin	27.48
KIRGTSNGT	ANTIGENIC	0.9599	NON-ALLERGEN	Non-Toxin	58.39
KMPGPGAYN	ANTIGENIC	0.4466	NON-ALLERGEN	Non-Toxin	40.19
KTRPFTLPN	ANTIGENIC	0.936	NON-ALLERGEN	Non-Toxin	76.71
LDGTPFHPF	ANTIGENIC	0.4336	NON-ALLERGEN	Non-Toxin	31.33
LDPIEVPLE	ANTIGENIC	0.7746	NON-ALLERGEN	Non-Toxin	96.52
LFLVPPTVE	ANTIGENIC	0.5709	NON-ALLERGEN	Non-Toxin	79.06
LGPHLNPFL	ANTIGENIC	0.6205	NON-ALLERGEN	Non-Toxin	64.48
LKPVGTASS	ANTIGENIC	0.8525	NON-ALLERGEN	Non-Toxin	82.26
LLHLSQMYN	ANTIGENIC	0.5305	NON-ALLERGEN	Non-Toxin	85.93
LPCLLPQEY#	ANTIGENIC	1.22	NON-ALLERGEN	Non-Toxin	98.71
LSLGPHLNP	ANTIGENIC	1.769	NON-ALLERGEN	Non-Toxin	74.71
LTELDGTPF	ANTIGENIC	0.5778	NON-ALLERGEN	Non-Toxin	95.93
MMMASKDAT	ANTIGENIC	0.7605	NON-ALLERGEN	Non-Toxin	18.74
MRVRIMLAG	ANTIGENIC	0.713	NON-ALLERGEN	Non-Toxin	56.45
NLPLSSLSN	ANTIGENIC	0.3874	NON-ALLERGEN	Non-Toxin	18.41
NMRVRIMLA	ANTIGENIC	0.7144	NON-ALLERGEN	Non-Toxin	84.98
NPFLLHLSQ#	ANTIGENIC	0.5239	NON-ALLERGEN	Non-Toxin	99.81
NVQSVQFQN	ANTIGENIC	1.476	NON-ALLERGEN	Non-Toxin	80.81
NYGSSITEA#	ANTIGENIC	0.6598	NON-ALLERGEN	Non-Toxin	99.43
NYVGVLSWI	ANTIGENIC	0.6615	NON-ALLERGEN	Non-Toxin	40.19
PCLLPQEYI	ANTIGENIC	0.9244	NON-ALLERGEN	Non-Toxin	49.64
PEVNASDPL	ANTIGENIC	0.496	NON-ALLERGEN	Non-Toxin	4.77
PFTLPNLPL	ANTIGENIC	0.7415	NON-ALLERGEN	Non-Toxin	17.84
PGDVLFDLS	ANTIGENIC	0.6402	NON-ALLERGEN	Non-Toxin	49.01

Epitope	Antigenicity	Threshold	Allergenicity	toxicity	PC%
PHLGSIQAN	ANTIGENIC	1.1721	NON-ALLERGEN	Non-Toxin	59.81
PISSMGISP	ANTIGENIC	0.9093	NON-ALLERGEN	Non-Toxin	4.77
PLEDVRNVL	ANTIGENIC	0.6153	NON-ALLERGEN	Non-Toxin	0
PLPISSMGI#	ANTIGENIC	0.8937	NON-ALLERGEN	Non-Toxin	97.59
PLSSLSNSR	ANTIGENIC	0.564	NON-ALLERGEN	Non-Toxin	34.55
PQGEFTISP	ANTIGENIC	1.0718	NON-ALLERGEN	Non-Toxin	65.74
PVGTASSAR	ANTIGENIC	0.4948	NON-ALLERGEN	Non-Toxin	18.41
PVSLSHVAK	ANTIGENIC	1.1364	NON-ALLERGEN	Non-Toxin	56.45
QNGRCTLDG	ANTIGENIC	0.9735	NON-ALLERGEN	Non-Toxin	35.12
QQTMRLVCM	ANTIGENIC	1.4154	NON-ALLERGEN	Non-Toxin	75.44
QSVQFQNGR	ANTIGENIC	0.835	NON-ALLERGEN	Non-Toxin	7.04
RAPLPISSM	ANTIGENIC	0.4337	NON-ALLERGEN	Non-Toxin	94.8
RFYQLKPVG	ANTIGENIC	0.4268	NON-ALLERGEN	Non-Toxin	49.64
RLVCMLYTP	ANTIGENIC	0.784	NON-ALLERGEN	Non-Toxin	43.75
RPFTLPNLP	ANTIGENIC	0.656	NON-ALLERGEN	Non-Toxin	68.56
RTLDPIEVP	ANTIGENIC	0.9334	NON-ALLERGEN	Non-Toxin	4.77
RVRIMLAGN	ANTIGENIC	0.4727	NON-ALLERGEN	Non-Toxin	61.89
SDPLAMDPV	ANTIGENIC	0.7145	NON-ALLERGEN	Non-Toxin	66.26
SHNLTIAQA	ANTIGENIC	0.535	NON-ALLERGEN	Non-Toxin	92.36
SNGTVINLT	ANTIGENIC	0.816	NON-ALLERGEN	Non-Toxin	65.74
SSLSNSRAP	ANTIGENIC	0.4709	NON-ALLERGEN	Non-Toxin	56.45
SSMGISPDN	ANTIGENIC	1.1512	NON-ALLERGEN	Non-Toxin	75.44
STAVATAGQ	ANTIGENIC	0.4945	NON-ALLERGEN	Non-Toxin	34.55
SVQFQNGRC	ANTIGENIC	0.7682	NON-ALLERGEN	Non-Toxin	56.45
SVYPPGFGE	ANTIGENIC	0.4006	NON-ALLERGEN	Non-Toxin	4.77
TAVATAGQV	ANTIGENIC	0.524	NON-ALLERGEN	Non-Toxin	93.03
TELDGTPFH	ANTIGENIC	0.6336	NON-ALLERGEN	Non-Toxin	92.59
TIAQATLFP	ANTIGENIC	0.4167	NON-ALLERGEN	Non-Toxin	45.16
TQFGHSSQT	ANTIGENIC	1.1401	NON-ALLERGEN	Non-Toxin	70.74
TQYDVDTTP	ANTIGENIC	0.8134	NON-ALLERGEN	Non-Toxin	43.67
VAGSSTAVA	ANTIGENIC	0.3894	NON-ALLERGEN	Non-Toxin	76.04
VATAGQVNP	ANTIGENIC	0.999	NON-ALLERGEN	Non-Toxin	0
VGEAALLHY	ANTIGENIC	0.5737	NON-ALLERGEN	Non-Toxin	54.08
VIADVRTLD	ANTIGENIC	1.0257	NON-ALLERGEN	Non-Toxin	49.64
VLFDLSLGP	ANTIGENIC	1.9011	NON-ALLERGEN	Non-Toxin	78.36
VPPTVEQKT#	ANTIGENIC	1.4434	NON-ALLERGEN	Non-Toxin	99.76
VQSVQFQNG	ANTIGENIC	1.09	NON-ALLERGEN	Non-Toxin	0
VYPPGFGEV	ANTIGENIC	0.5505	NON-ALLERGEN	Non-Toxin	77.62
WVGNMRVRI	ANTIGENIC	0.7801	NON-ALLERGEN	Non-Toxin	85.85
YDVDTTPDT	ANTIGENIC	0.6213	NON-ALLERGEN	Non-Toxin	82.42
YGSSITEAT	ANTIGENIC	0.5112	NON-ALLERGEN	Non-Toxin	66.37
YNGWVGNMR	ANTIGENIC	0.4048	NON-ALLERGEN	Non-Toxin	85.21
YPPGFGEVL#	ANTIGENIC	0.4272	NON-ALLERGEN	Non-Toxin	84.37
YQLKPVGTA	ANTIGENIC	1.6335	NON-ALLERGEN	Non-Toxin	95.53
YVGVLSWIS	ANTIGENIC	0.7008	NON-ALLERGEN	Non-Toxin	58.52

PC: population coverage
#the proposed epitopes.

Among the 105 epitopes, eight epitopes namely ($_{216}$FLFLVPPTV$_{224}$; $_{499}$GVFVFVSWV$_{507}$; $_{433}$LPCLLPQEY$_{441}$; $_{90}$NPFLLHLSQ$_{98}$; $_{394}$NYGSSITEA$_{402}$; $_{247}$PLPISSMGI$_{255}$; $_{220}$VPPTVEQKT$_{228}$; $_{410}$YPPGFGEVL$_{418}$) were interacted with most frequent MHC class II alleles, demonstrated higher population coverage and three of them were shown to interact with both MHC class I and MHC class II alleles. Therefore they were proposed as T helper cells epitopes. The eight epitopes and their corresponding MHC-II alleles were shown in Table 7. The position of these predicted epitopes in the 3D structural level in the capsid protein was illustrated in Figure 5 and Figure 6.

Table 7. The best eight epitopes that were proposed as a vaccine candidate from the capsid protein (VP1) of the Norwalk virus and interacted with high affinity with MHC class II alleles. The position of epitopes is according to the position of amino acids in the capsid protein

Core Sequence	Peptide Sequence	Start	End	Allele	IC50	Rank
FLFLVPPTV	DFNFLFLVPPTVEQK	213	227	HLA-DRB1*01: 01	3.9	0.05
				HLA-DRB1*04: 01	156.5	12.23
				HLA-DRB1*04: 05	18.4	1.1
				HLA-DRB1*08: 02	745.7	17.59

Core Sequence	Peptide Sequence	Start	End	Allele	IC50	Rank
				HLA-DRB1*09: 01	111.5	7.67
				HLA-DRB1*11: 01	61.1	9.89
				HLA-DRB1*13: 02	366.5	14.43
				HLA-DRB1*15: 01	81.3	8.26
				HLA-DRB4*01: 01	735.2	34.43
				HLA-DRB5*01: 01	326.7	27.61
				HLA-DQA1*01: 01/DQB1*05: 01	608.9	10.46
				HLA-DQA1*05: 01/DQB1*02: 01	348.7	7.88
				HLA-DQA1*05: 01/DQB1*03: 01	1232.7	52.02
	FLFLVPPTVEQKTRP	216	230	HLA-DRB1*01: 01	4.9	0.71
				HLA-DRB1*04: 04	633.1	38.92
				HLA-DRB1*04: 05	69.4	6.84
				HLA-DRB1*08: 02	1202.5	25.7
				HLA-DRB1*09: 01	232.1	14.57
				HLA-DRB1*11: 01	159	17.95
				HLA-DRB1*13: 02	1003.5	25.96
				HLA-DRB1*15: 01	200.8	17.01
				HLA-DRB4*01: 01	828.8	36.76
				HLA-DRB5*01: 01	587.5	35.29
				HLA-DQA1*05: 01/DQB1*02: 01	945.4	19.55
				HLA-DQA1*05: 01/DQB1*03: 01	1861.5	60.33
				HLA-DPA1*01/DPB1*04: 01	1772.4	29.37
	FNFLFLVPPTVEQKT	214	228	HLA-DRB1*01: 01	3.9	0.05
				HLA-DRB1*04: 01	167.7	12.97
				HLA-DRB1*04: 05	22.2	1.54
				HLA-DRB1*08: 02	640.4	15.35
				HLA-DRB1*09: 01	127	8.67
				HLA-DRB1*11: 01	69.8	10.87
				HLA-DRB1*13: 02	462	16.61
				HLA-DRB1*15: 01	92.8	9.31
				HLA-DRB4*01: 01	443.3	25.38
				HLA-DRB5*01: 01	318.9	27.32
				HLA-DQA1*01: 01/DQB1*05: 01	889.1	13.7
				HLA-DQA1*05: 01/DQB1*02: 01	528.5	11.76
				HLA-DQA1*05: 01/DQB1*03: 01	1296.9	53.02
	NFLFLVPPTVEQKTR	215	229	HLA-DRB1*01: 01	4.3	0.25
				HLA-DRB1*04: 05	36.8	3.33
				HLA-DRB1*08: 02	654.6	15.65
				HLA-DRB1*09: 01	158.8	10.6
				HLA-DRB1*11: 01	82.4	12.15
				HLA-DRB1*13: 02	588.8	19.16
				HLA-DRB1*15: 01	96.3	9.61
				HLA-DRB4*01: 01	546	28.96
				HLA-DRB5*01: 01	354.9	28.61
				HLA-DQA1*01: 01/DQB1*05: 01	1620.6	20.31
				HLA-DQA1*05: 01/DQB1*02: 01	805.3	17.12
				HLA-DQA1*05: 01/DQB1*03: 01	1440	55.12
				HLA-DPA1*01/DPB1*04: 01	1466	26.63
	PDFNFLFLVPPTVEQ	212	226	HLA-DRB1*01: 01	4	0.09
				HLA-DRB1*04: 01	177.5	13.59
				HLA-DRB1*04: 05	15.8	0.8
				HLA-DRB1*08: 02	1076.2	23.64
				HLA-DRB1*09: 01	121.3	8.31
				HLA-DRB1*11: 01	95.4	13.34
				HLA-DRB1*13: 02	367.8	14.47
				HLA-DRB1*15: 01	86.3	8.71
				HLA-DRB3*01: 01	1985.4	27.39
				HLA-DRB4*01: 01	871.2	37.78
				HLA-DRB5*01: 01	558.5	34.57
				HLA-DQA1*01: 01/DQB1*05: 01	530.8	9.45

Core Sequence	Peptide Sequence	Start	End	Allele	IC50	Rank
				HLA-DQA1*05: 01/DQB1*02: 01	304.2	6.84
				HLA-DQA1*05: 01/DQB1*03: 01	1538	56.44
	PSPDFNFLFLVPPTV	210	224	HLA-DRB1*01: 01	4.4	0.32
				HLA-DRB1*04: 01	264.2	18.58
				HLA-DRB1*04: 05	17.6	1
				HLA-DRB1*07: 01	256.7	24.31
				HLA-DRB1*08: 02	2285.2	39.25
				HLA-DRB1*09: 01	166.4	11.08
				HLA-DRB1*11: 01	225.3	21.55
				HLA-DRB1*13: 02	997.6	25.88
				HLA-DRB1*15: 01	134.3	12.68
				HLA-DRB4*01: 01	1237.8	45.06
				HLA-DRB5*01: 01	1436	49.13
				HLA-DQA1*01: 01/DQB1*05: 01	572.2	9.99
				HLA-DQA1*05: 01/DQB1*02: 01	329.7	7.44
				HLA-DQA1*05: 01/DQB1*03: 01	2038.4	62.22
	SPDFNFLFLVPPTVE	211	225	HLA-DRB1*01: 01	4.2	0.19
				HLA-DRB1*04: 01	246.8	17.63
				HLA-DRB1*04: 05	15.4	0.77
				HLA-DRB1*07: 01	247.1	23.86
				HLA-DRB1*08: 02	1387.9	28.52
				HLA-DRB1*09: 01	141.4	9.57
				HLA-DRB1*11: 01	135.5	16.44
				HLA-DRB1*13: 02	408.9	15.41
				HLA-DRB1*15: 01	107.1	10.52
				HLA-DRB3*01: 01	1646.7	24.33
				HLA-DRB4*01: 01	1019.7	40.96
				HLA-DRB5*01: 01	900.5	41.61
				HLA-DQA1*01: 01/DQB1*05: 01	509.2	9.16
				HLA-DQA1*05: 01/DQB1*02: 01	294.1	6.61
				HLA-DQA1*05: 01/DQB1*03: 01	1760.9	59.18
GVFVFVSWV	GVFVFVSWVSRFYQL	499	513	HLA-DRB4*01: 01	2040.8	56.23
	INGVFVFVSWVSRFY	497	511	HLA-DQA1*01: 01/DQB1*05: 01	616.7	10.55
	LPINGVFVFVSWVSR	495	509	HLA-DQA1*01: 01/DQB1*05: 01	1527.6	19.59
	NGVFVFVSWVSRFYQ	498	512	HLA-DRB4*01: 01	2723.6	62.82
				HLA-DQA1*01: 01/DQB1*05: 01	486.5	8.87
	PINGVFVFVSWVSRF	496	510	HLA-DQA1*01: 01/DQB1*05: 01	988.2	14.74
	QLPINGVFVFVSWVS	494	508	HLA-DQA1*01: 01/DQB1*05: 01	1570.8	19.93
				HLA-DQA1*05: 01/DQB1*02: 01	2253.4	36.31
				HLA-DPA1*01: 03/DPB1*02: 01	242.1	14.4
	QQLPINGVFVFVSWV	493	507	HLA-DQA1*01: 01/DQB1*05: 01	2281.7	25.02
				HLA-DPA1*01/DPB1*04: 01	2890.9	37.51
				HLA-DPA1*01: 03/DPB1*02: 01	1279.9	34.28
LPCLLPQEY	AYNLPCLLPQEYISH	430	444	HLA-DRB4*01: 01	273.7	18.22
				HLA-DQA1*05: 01/DQB1*02: 01	893	18.65
	GAYNLPCLLPQEYIS	429	443	HLA-DRB4*01: 01	334.1	21
				HLA-DQA1*05: 01/DQB1*02: 01	568.4	12.59
	GPGAYNLPCLLPQEY	427	441	HLA-DRB4*01: 01	942.9	39.34
				HLA-DQA1*05: 01/DQB1*02: 01	380.9	8.61
	LPCLLPQEYISHLAS	433	447	HLA-DQA1*05: 01/DQB1*02: 01	1576.5	28.7
	NLPCLLPQEYISHLA	432	446	HLA-DQA1*05: 01/DQB1*02: 01	1251.9	24.29
	PGAYNLPCLLPQEYI	428	442	HLA-DRB4*01: 01	434.7	25.06
				HLA-DQA1*05: 01/DQB1*02: 01	401.3	9.06
	YNLPCLLPQEYISHL	431	445	HLA-DRB4*01: 01	196.8	14.12
				HLA-DQA1*05: 01/DQB1*02: 01	819.2	17.37
NPFLLHLSQ	GPHLNPFLLHLSQMY	86	100	HLA-DRB1*11: 01	254.6	22.9
				HLA-DRB4*01: 01	166.1	12.28
	HLNPFLLHLSQMYNG	88	102	HLA-DRB1*11: 01	166.9	18.44
				HLA-DRB4*01: 01	124.7	9.56
	LGPHLNPFLLHLSQM	85	99	HLA-DRB1*11: 01	450	29.68
	PHLNPFLLHLSQMYN	87	101	HLA-DRB1*11: 01	163.4	18.22
				HLA-DRB4*01: 01	129.7	9.9
	SLGPHLNPFLLHLSQ	84	98	HLA-DRB1*11: 01	691.2	35.43
				HLA-DRB4*01: 01	561.6	29.46

Core Sequence	Peptide Sequence	Start	End	Allele	IC50	Rank
NYGSSITEA	DLWKIPNYGSSITEA	388	402	HLA-DQA1*01: 02/DQB1*06: 02	438.1	25.11
				HLA-DQA1*04: 01/DQB1*04: 02	1312	20.23
				HLA-DQA1*05: 01/DQB1*03: 01	114.7	16.23
				HLA-DPA1*02: 01/DPB1*01: 01	2907.3	62.53
	IPNYGSSITEATHLA	392	406	HLA-DQA1*01: 02/DQB1*06: 02	114	8.22
				HLA-DQA1*03: 01/DQB1*03: 02	1910.7	30.8
				HLA-DQA1*04: 01/DQB1*04: 02	1777.9	26.07
				HLA-DPA1*02: 01/DPB1*01: 01	2635.9	60.65
	KIPNYGSSITEATHL	391	405	HLA-DQA1*01: 02/DQB1*06: 02	98.4	7
				HLA-DQA1*03: 01/DQB1*03: 02	1839.4	29.9
				HLA-DQA1*04: 01/DQB1*04: 02	1484.4	22.49
	LWKIPNYGSSITEAT	389	403	HLA-DQA1*01: 02/DQB1*06: 02	131.9	9.55
				HLA-DQA1*03: 01/DQB1*03: 02	1901.2	30.69
				HLA-DQA1*04: 01/DQB1*04: 02	1175.6	18.36
	NYGSSITEATHLAPS	394	408	HLA-DQA1*01: 02/DQB1*06: 02	98.2	6.98
				HLA-DQA1*03: 01/DQB1*03: 02	2319	35.58
	PNYGSSITEATHLAP	393	407	HLA-DQA1*01: 02/DQB1*06: 02	141	10.19
				HLA-DQA1*03: 01/DQB1*03: 02	2259.1	34.92
				HLA-DQA1*04: 01/DQB1*04: 02	2664.1	35.49
	WKIPNYGSSITEATH	390	404	HLA-DQA1*01: 02/DQB1*06: 02	101.1	7.21
				HLA-DQA1*03: 01/DQB1*03: 02	1776.1	29.08
				HLA-DQA1*04: 01/DQB1*04: 02	1305.8	20.15
PLPISSMGI	APLPISSMGISPDNV	246	260	HLA-DRB1*01: 01	636.5	60.52
				HLA-DRB1*07: 01	1251.1	48.06
				HLA-DRB1*13: 02	791	22.72
				HLA-DRB1*15: 01	1327.3	45.02
				HLA-DRB4*01: 01	132.9	10.13
	LSNSRAPLPISSMGI	241	255	HLA-DRB1*01: 01	182.5	39.35
				HLA-DRB1*07: 01	132.3	16.9
				HLA-DRB1*13: 02	330.5	13.54
				HLA-DRB1*15: 01	1295.9	44.6
				HLA-DRB4*01: 01	409.1	24.09
	NSRAPLPISSMGISP	243	257	HLA-DRB1*01: 01	171.5	38.32
				HLA-DRB1*07: 01	489.8	32.98
				HLA-DRB1*13: 02	551.1	18.44
				HLA-DRB1*15: 01	834.1	37.09
				HLA-DRB4*01: 01	309.5	19.9
	PLPISSMGISPDNVQ	247	261	HLA-DRB1*07: 01	1504.5	51.34
				HLA-DRB1*13: 02	865.8	23.92
				HLA-DRB1*15: 01	2068.6	53.02
				HLA-DRB4*01: 01	120.4	9.26
	RAPLPISSMGISPDN	245	259	HLA-DRB1*01: 01	341.7	50.41
				HLA-DRB1*07: 01	982.5	43.9
				HLA-DRB1*13: 02	711.4	21.37
				HLA-DRB1*15: 01	976.9	39.72
				HLA-DRB4*01: 01	134.6	10.24
	SNSRAPLPISSMGIS	242	256	HLA-DRB1*01: 01	131.4	34.14
				HLA-DRB1*07: 01	200.4	21.42
				HLA-DRB1*13: 02	518.7	17.78
				HLA-DRB1*15: 01	915.8	38.65
				HLA-DRB4*01: 01	308.5	19.86
	SRAPLPISSMGISPD	244	258	HLA-DRB1*01: 01	186	39.66
				HLA-DRB1*07: 01	667.6	37.67
				HLA-DRB1*13: 02	536.4	18.15
				HLA-DRB1*15: 01	781.7	36.04
				HLA-DRB4*01: 01	171.7	12.64
VPPTVEQKT	FLVPPTVEQKTRPFT	218	232	HLA-DRB4*01: 01	2262.6	58.61
	LFLVPPTVEQKTRPF	217	231	HLA-DRB4*01: 01	1715.7	52.27
	LVPPTVEQKTRPFTL	219	233	HLA-DRB4*01: 01	1665.6	51.62
	VPPTVEQKTRPFTLP	220	234	HLA-DRB4*01: 01	1603.9	50.77

Core Sequence	Peptide Sequence	Start	End	Allele	IC50	Rank
YPPGFGEVL	APSVYPPGFGEVLVF	406	420	HLA-DRB1*01: 01	785.9	63.71
				HLA-DQA1*05: 01/DQB1*03: 01	236.8	24.74
	HLAPSVYPPGFGEVL	404	418	HLA-DQA1*05: 01/DQB1*02: 01	2445.4	38.19
				HLA-DQA1*05: 01/DQB1*03: 01	368	31.01
	LAPSVYPPGFGEVLV	405	419	HLA-DQA1*05: 01/DQB1*02: 01	2186.5	35.63
				HLA-DQA1*05: 01/DQB1*03: 01	306.8	28.35
	PSVYPPGFGEVLVFF	407	421	HLA-DRB1*01: 01	287.5	47.37
				HLA-DRB1*15: 01	2754.8	58.37
				HLA-DQA1*05: 01/DQB1*03: 01	167.8	20.43
	SVYPPGFGEVLVFFM	408	422	HLA-DQA1*05: 01/DQB1*03: 01	176.5	21.04
	VYPPGFGEVLVFFMS	409	423	HLA-DQA1*05: 01/DQB1*03: 01	179	21.2
	YPPGFGEVLVFFMSK	410	424	HLA-DQA1*05: 01/DQB1*03: 01	197.4	22.41

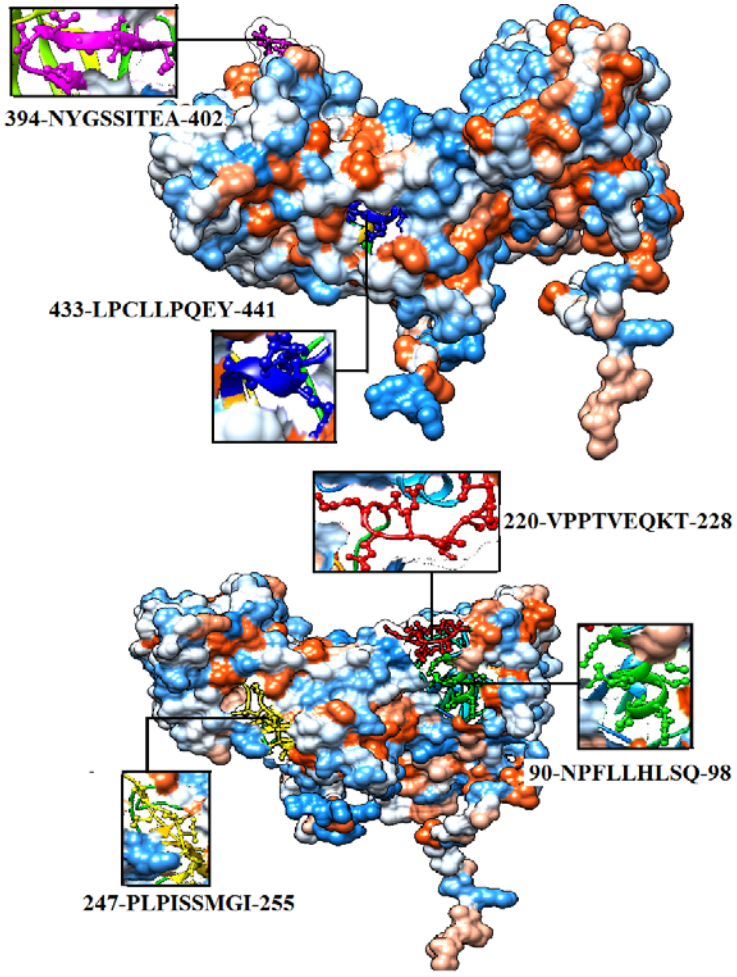

Figure 6. T cell proposed epitopes that interact with MHC-1I alleles. Three epitopes ($_{216}$-FLFLVPPTV-$_{224}$; $_{499}$-GVFVFVSWV-$_{507}$ and $_{410}$-YPPGFGEVL-$_{418}$) were not shown in this figure since they were shown in figure (5) (interacted with MHC-1 and MHC-1I alleles). The other five epitopes (433-LPCLLPQEY-441; 90-NPFLLHLSQ-98; 394-NYGSSITEA-402; 247-PLPISSMGI-255 and 220-VPPTVEQKT-228) were only shown in this figure since they only interacted with MHC-1I alleles. The positions of these proposed epitopes was according to their position in the capsid protein

3.6. Analysis of the Population Coverage

The predicted epitopes from the capsid protein (VP1) that interacting with MHC class I and II alleles were subjected to population coverage analysis. The proposed vaccine epitopes were elected as peptide vaccine based on their high population coverage score, number of their interacted alleles and/or their interaction with both MHC class I and II alleles. As shown in (Table 4) 22 predicted epitopes interacted with MHC class I alleles with different population coverage scores. Among them four epitopes scored high population coverage % and highly interacted with most frequent MHC class I alleles. This strengthen their potentiality to act as promising vaccine candidate against MHC class I therefore were proposed as peptide vaccine. The epitope set of these four epitopes against MHC-1 alleles was 60.35% (Table 8).

In addition to that, as shown in Table 6 MHC class II, 105 predicted epitopes interacted with MHC class II alleles with different population coverage scores and were shown to be antigenic, nonallergic and nontoxic. Among them eight epitopes highly interacted with most frequent MHC class II alleles therefore they were proposed as peptide vaccine.

Table 8. The population coverage (PC) of MHC-I and MHC-II proposed epitopes. The population coverage of MHC-I was 80.53%, MHC-II was 99.99% and the combined alleles was 100% for all proposed epitopes)PC: Population Coverage)

MHC-I		MHC-II		COMBINED MHC-I/MHC-II	
Epitope	PC%	Epitope	PC%	Epitope	PC%
FLFLVPPTV	46.73	FLFLVPPTV	88.87	FLFLVPPTV	98.14
GVFVFVSWV	40.6	GVFVFVSWV	79.44	GVFVFVSWV	97.03
YPPGFGEVL	26.51	YPPGFGEVL	84.37	YPPGFGEVL	83.55
AQATLFPHV	45.62	PLPISSMGI	97.59	PLPISSMGI	97.59
Epitope set	60.35%	LPCLLPQEY	98.71	LPCLLPQEY	98.71
		NPFLLHLSQ	99.81	NPFLLHLSQ	99.81
		NYGSSITEA	99.43	NYGSSITEA	99.43
		VPPTVEQKT	99.76	PLPISSMGI	97.59
		Epitope set	99.96%	AQATLFPHV	45.62
				Epitope set	100%

The epitope set of these eight epitopes against MHC-II alleles was 99.96% (Table 8). Furthermore as shown in Table 8 all proposed epitopes were subjected to population coverage tools to assess population coverage of their MHC-I and MHC-II combined alleles. The population coverage of the proposed epitopes against the combined alleles was 100%.

4. Discussion

Norwalk viruses are considered as a leading etiology of epidemic acute gastroenteritis as well as an important cause of sporadic cases of acute gastroenteritis [42]. Currently, there is no vaccine to prevent human norovirus infection, and there is no specific therapy available to treat it [20]. The norovirus genome has three open reading frames (ORFs) of which ORF2 and ORF3 encode the major capsid protein (VP1) that determines the antigenicity of the virus, as well as the minor capsid protein (VP2). The majority of the studies performed to design vaccine for norovirus used VP1 as a vaccine construct. For instance vaccine using recombinant adenovirus expressing the norovirus major capsid protein VP1was developed. The vaccine measured the cross-reactive neutralizing antibody responses which are required for a successful norovirus vaccine [43]. A study by Tucker et al (2008) [44] developed a currently human clinical trials vaccine that employs a recombinant adenovirus expressing the norovirus GI.1 major capsid protein (VP1) in an oral tablet formulation developed by Vaxart, Inc. Moreover recombinant adenovirus vaccine expressing the norovirus GII.4 major capsid protein VP1 [45] and multiple VLP vaccine developed from VP1 protein [46] were developed by the Chinese center for disease control and prevention. Both studies demonstrated the antigenicity of VP1 as a vaccine candidate. Therefore this study aimed to propose multiple epitopes vaccine candidates from the capsid protein (VP1) to elicit B and T lymphocytes and act as a vaccine candidate using immune-informatics tools.

In the current study the B cell epitopes were predicted from the capsid protein VP1 tofind the potential epitopes that would interact with B lymphocytes and initiate immune response. For the vaccine to be recognized by the B cell antibodies it must be linear and located on the surface of the antigen protein to be easily accessible. In addition to that the candidate vaccine should demonstrate greater antigenicity to elicit antibodies production. Therefore several tools from IEDB analysis resources were used to identify B cell epitopes such as Bepipred linear epitope prediction analysis [33], Emini surface accessibility prediction [34] and Kolaskar and Tongaonkar antigenicity scale [35]. These tools provided multiple epitopes that were linear, on the protein surface and antigenic. However, for the epitopes to be proposed as a vaccine candidate it should be nonallergic and nontoxic to the host cells [47]. Thus the predicted epitopes further subjected to Vaxigen antigenicity, allergenicity and toxicity investigations. Eight epitopes eight epitopes namely $_{48}$QVNP$_{51}$, $_{159}$EVPLE$_{163}$, $_{224}$VEQK$_{227}$, $_{245}$RAPLP$_{249}$, $_{376}$ISPPS$_{380}$, $_{409}$VYPP$_{412}$, $_{473}$FKAY$_{476}$ and $_{492}$PQQLP$_{496}$ passed these criteria and proposed as a B cell epitopes.

Since the immune response of T cell is long lasting response compared to B cell, where the antigen can easily escape the antibody memory response. This considered that CD8+T and CD4+T cells response play a major role in antiviral immunity [48]. Cytotoxic CD8+T lymphocytes are considered as an important parameter in recognizing and killing infected cells or producing specific cytokines that prevent the infection in the body [49,50]. Thus, T cell epitope-based vaccination is a unique process of eliciting strong immune response against infectious agents such as viruses [51]. In this study four epitopes were shown to interact with Cytotoxic CD8+T lymphocytes with high number of MHC-1 alleles and they demonstrated antigenicity and were shown to be nonallergenic and nontoxic. Beside that the four proposed epitopes showed favourable population coverage against the whole world population with epitopes set 60.35%. Therefore were proposed as Cytotoxic CD8+T lymphocytes epitopes.

For Helper CD4+T lymphocytes eight epitopes namely $_{90}$-NPFLLHLSQ-$_{98}$; $_{216}$-FLFLVPPTV-$_{224}$; $_{499}$-GVFVFVSWV-$_{507}$; $_{433}$-LPCLLPQEY-$_{441}$; $_{394}$-NYGSSITEA-$_{402}$; $_{247}$-PLPISSMGI-$_{255}$; $_{220}$-VPPTVEQKT-$_{228}$; $_{410}$-YPPGFGEVL-$_{418}$ were shown to interact with Helper CD4+T lymphocytes with high number of MHC-11 alleles and they were shown to be antigenic, nonallergenic and nontoxic. Among the eight epitopes three epitopes namely $_{216}$-FLFLVPPTV-$_{224}$; $_{499}$-GVFVFVSWV-$_{507}$ and $_{410}$-YPPGFGEVL-$_{418}$ were shown to interact with both MHC-1 and MHC-11 alleles. This favoured them to be elected as a vaccine candidate. Also the eight epitopes demonstrated favourable interaction with the whole world population coverage with epitopes

set 99.96%. Therefore were proposed as Helper CD4+T lymphocytes epitopes. The overall epitope set for the MHC-I and MHC-II combined alleles for the proposed epitopes showed excellent population coverage against whole world population (100%). Accordingly these epitopes were strongly recommended as promising epitopes vaccine candidates against T lymphocyte cells.

Recently a study by Azim et al (2019) [47] used multiple bioinformatics tools to predict epitopes from the VP1 and VP2 proteins of the noroviruse. However none of their predicted epitopes for B and T lymphocytes were corroborated to our proposed epitopes. This might be attributed to the differences in the software used in both studies to predict vaccine candidates.

5. Conclusion

The developing of an effective and safe vaccine is recommended to combat the infection and noroviruses. Vaccine design using reverse vaccinology prediction methods is highly appreciated as it provided specific epitopes in protein to act as a vaccine without using the virus particles as components of the vaccine. In this study eight epitopes were successfully proposed to interact against B cells. Moreover nine epitopes were successfully predicted to interact against T cell with population coverage epitope set of 100%. These epitopes provided excellent results as promising vaccine against noroviruses. However in vitro and in vivo trials are required to achieve the effectiveness of these epitopes as vaccine candidates.

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Competing Interest

The authors declared that they have no competing interests.

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