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In silico Prediction of Peptide based Vaccine against Fowlpox Virus (FPV)

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Abstract

Fowlpox virus (FPV) is double stranded DNA virus and a member of *Poxviridae* family which transmitted *via* aerosols and insect bite and causes cutaneous and diphtheritic infection in poultry population. This study aimed to design peptide vaccine by selecting all possible epitopes after analyzing of all FPV140 protein sequence reported in NCBI database using *in silico* approaches. After alignment of retrieved sequence the conserved region applied into IEDB analysis tool to predict B and T cell epitopes, then testing the affinity of predicted epitopes to bind to (BF2*2101) (BF2*0401) chicken receptor for MHC1 molecule, peptides low energy when docked against receptor were suggested as epitopes based vaccine. Peptides (50 PPSPKP 55, 51 PSPKPL 56, 52 SPKPLP 57, 53 PKPLPK 58, 54 KPLPKS 59, 55 PLPKSK 60, 56 LPKSKQ 61 and 18 RPSSTV 23) were most potential B cell epitopes while (110 YIMDNAEKL 118, 274 FYHRMYYPL 282, 278 MYYPLFSVF 286 231 YVVDNDRYV 239 and 317 LLSGVFLAY 325) docked epitopes suggested to be T cell epitopes because of their good binding affinity especially this overlapped one 110 YIMDNAEKL 118. This study concluded that those predicted epitopes might use to produce good vaccine against FPV after *in vitro* and *in vivo* studies to evaluate its efficiency.

Keywords: Fowlpox virus; Epitopes; Vaccine; Insect bite

Introduction

Fowlpox virus (FPV) is a worldwide spread virus and high prevalent in tropical and subtropical countries. It's highly infectious but slow in spreading. The occurrence of infection is variable according to climates, hygiene and vaccination. FPV infects chickens, turkeys and other type of birds mindless of differences in sex, age and breed it transmitted directly from infected birds by inhalation or indirectly by insect bites. It causes two type of infection dry pox (mild) or wet pox (severe) infection. The dry type also known as cutaneous infection is featured by lesions or nodules on unfeathered areas of the bird body. This form has high currency but it's mild. The severe form is the wet type known as diphtheritic infection which infects mucus membrane of respiratory and gastrointestinal tract especially (larynx, pharynx and mouth) is featured by necrotic lesions, this type cause death more than dry type due to suffocation [1-16].

FPV related to genus *Avipox* (APVs). APVs belong to subfamily *Chordopoxvirinae* which is the part of *Poxviridae* family. APVs are large, oval shaped enveloped viruses with double strand DNA. APVs differ from other DNA viruses, they replicate simply in cytoplasm. The mature FPV is brick like shape, with dimension 330 × 280 × 200 nm. The outer membrane contain random package of surface tubules. The virion composed of biconcave nucleoid in the center with two bodies in sides DNA of FPV consists of 288-300 kilo base pair. FPV140 is one of membrane associate protein of FPV the protein functions in attachment of intracellular mature virus particles (IMVP) to cell. It's used to differentiate FPV from other APVs because it's highly conserved. FPV140 is highly antigenic and immunodominent [1-4,6,8-15,17,18].

FPV survives for long time in poultry environment in contrast to other viruses because its genome contains genes which protect it from environment (photolyase and A type inclusion body genes). FPV disease lead to severe economic crash globally which result from plunge in egg production, reduction in growth of young birds, blindness and in some cases death [1,5,6,9,12-15].

Vaccines activate body resistance to specific diseases by starting the immunological reaction and decrease clinical signs and downturn virus shedding and transmission. Vaccines for chickens are usually inactivated vaccines which are time consuming, labor intensive, expensive and inaccurate or live vaccines which are widely used but it can cause disease depending on the environmental factors and immunity status it's recently improved by genetic modification but the high cost is obstacles. For FPV live vaccine is mainly used [8,19].

Epitope based vaccine depend on identification of specific epitopes from pathogen. These epitopes are capable of inducing B cell and T cell mediated immunity. Many studies show the effectiveness of peptide based vaccine against infectious disease such as malaria, HIV, TB and Hepatitis B. The insilico tools make the epitope prediction simple and easy, minimize the cost of construction and production of vaccine so that prevent infection hazards and eliminate the allergic and reactogenic response though it seems promising in next vaccine technology [19-22].

This study aimed to design peptide vaccine against FPV by using FPV 140 protein as target. No previous reports found in FPV epitopes based vaccine so this may considered the first study using insilico approach to design epitope vaccine against FPV which its outbreaks cause severe economic loss in poultry population.

Materials and Methods

Protein sequence retrieval

A total of 20 virulent strain of Fowl pox virus FPV140 protein

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were retrieved from NCBI (http://www.ncbi.nlm.nih.gov/protein/) database in Septemeber 2016. These 20 strains retrieved were selected from different parts of the world for immunobioinformtics analysis; retrieved protein strains and their accession numbers as well as date and area of collection are listed in Table 1.

Determination of conserved regions

The retrieved sequences were aligned to obtain conserved regions using multiple sequence alignment (MSA). Sequences aligned with the aid of ClustalW as implemented in the BioEdit program for finding the conserved regions among international virulent variants. Later on, the candidate epitopes were analyzed by different prediction tools from Immune Epitope Database IEDB analysis resource (http://www.iedb.org/) [23,24].

Sequence based methods

B-cell epitope prediction: B cell epitope is the portion of an immunogen, which interacts with B-lymphocytes. As a result, the B-lymphocyte is differentiated into antibody-secreting plasma cell and memory cell. B cell epitope is characterized by being hydrophilic and accessible [25] .Thus, the classical propensity scale methods and hidden Markov model programmed softwares from IEDB analysis resource were used for:

Prediction of linear B-cell epitopes: Depening on the following aspects: BepiPred from immune epitope database [26] was used for linear B-cell epitopes prediction from the conserved region with a default threshold value of 0.35.

Prediction of surface accessibility: By using Emini surface accessibility prediction tool of the immune epitope data base (IEDB) [27] the surface accessible epitopes were predicted from the conserved region holding the default threshold value 1.000 or higher.

Prediction of epitopes antigenicity sites: The kolaskar and tongaonker antigenicity method were used to determine the antigenic sites with a default threshold value of 1.042 [28].

Prediction of epitopes hydrophilicity: Parker hydrophilicity prediction tool was used to determine the hydrophilicity of the conserved regions; and the threshold default value was 1.286 [29].

Accession Number	Date of Collection	Country
NP-039103	NA	NA
AAF44484	1999	USA
AEB40184	2009	India
AEB40181	2008	India
AEB40178	2008	India
AEB40175	2008	India
AEB40172	2008	India
AEB40169	2008	India
AFS52252	2011	Egypt
AFS52251	2011	Egypt
AFS52250	2011	Egypt
AFS52249	2011	Egypt
CAJ21219	NA	United Kingdom
CAJ21216	NA	United Kingdom
CAJ21213	NA	United Kingdom
CAJ21210	NA	United Kingdom
CCA65952	NA	Austria
CCA65949	NA	Austria
Q9J590	NA	NA
ADP92335	NA	China

Table 1: Virus Strains retrieved and their Accession numbers and area of collection; *NA: not available.

T cell epitope prediction: It was done by online immune-informatics tool IEDB (http://tools.iedb.org). Prediction for several organisms is supported by this tool as chicken is not among them. However, several studies suggest some similarities between HLA alleles and chicken MHC, [30-34], So for MHC class-I and MHC class-II the man HLA A, B and C and HLA DR, DP and DQ were used respectively.

MHC class I binding predictions: The major histocompatibility complex MHC class-I binding prediction tool (http://tools.iedb.org/mhci/) [35] was used to predict Cytotoxic T cell epitopes. Prediction methods achieved by artificial neural network (ANN). Prior to prediction, all epitope lengths were set as 9 m, conserved epitopes that bind to many HLA alleles at score equal or less than 1.0 percentile rank and 100 IC50 were selected for further analysis [36].

MHC class II binding predictions: The MHC class-II binding prediction tool (http://tools.iedb.org/mhcii/) [37] was used to predict helper T-cell epitopes. The prediction achieved by NN- align that uses the artificial neural networks that allows for simultaneous identification of the MHC class II binding core epitopes and binding affinity. The percentile rank for strong binding peptides was set at ≤ 10 with IC50 ≤ 500 to determine the interaction potentials of helper T-cell epitope peptide and MHC class II allele (HLA DR, DP and DQ) [38]. All conserved epitopes that bind to many alleles at score equal or less than 10 percentile rank with IC50 ≤ 500 is selected for further analysis.

Structure-based methods

Homology modeling and visualization: FPV140 protein 3D structure obtained by phyre2, (http://www.sbg.bio.ic.ac.uk/phyre2) which uses advanced remote homology detection methods to build 3D models not as chicken alleles BF2 *2101 and BF2*0401 were retrieved from the NCBI database/structure (MMDB ID: 61647/PDB ID: 3BEW and MMDB ID: 105232/PDB ID 4G42, respectively) [39]. UCSF Chimera (version 1.8) was used to visualize the 3D structures, Chimera currently available within the Chimera package and available from the chimera web site (http://www.cgl.ucsf.edu/cimera). Homology modeling was achieved to establish docking, and for further verification of the service accessibility and hydrophilicity of B lymphocyte epitopes predicted, as well as to visualize all predicted T cell epitopes in the structural level [40,41].

Docking: Top epitopes of MHC I alleles that predicted to bind with IC50 below 100 and percentile rank less than 1.00 were selected as the ligands, which are modeled using PEP- FOLD online peptide modeling tool. Two chicken BF alleles /receptors (BF2 *2101, BF2*0401) have been evaluated according to peptide-binding groove affinity which reported by Kokh et al. [42] and Zhang et al. [43]. Protein sequence and PDB ID of BF2 *2101, BF2*0401 were retrieved from the NCBI database/structure (MMDB ID: 61647/PDB ID: 3BEW and MMDB ID: 105232/PDB ID 4G42, respectively) [44]. Molecular Docking technique applied by PatchDock (http://bioinfo3d.cs.tau.ac.il/PatchDock/) online auto-dock tools [44]. Then the visualization had done by UCSF-Chimera visualization tool 1.8 [45-48].

Results

B cell prediction and modelling

Sequences of FPV140 protein were applied to Bepipred linear epitope prediction, Emini surface accessibility, Kolaskar and Tongaonkar antigenicity and Parker hydrophobicity prediction tools in

IEDB. Eight B cell epitopes were predicted by Bepipred linear epitope prediction (Table 2).

There was eight epitopes succeeded the three test from those predicted epitopes 34 WSYKKGIKNGYDDYRDPPSPKPLPKSKQEPNADDKVGDIE 73 and 17 GRPSSTVV 24 (Table 3 and Figure 1).

Prediction of cytotoxic T cell epitopes and modelling

The reference FPV140 protein sequence was analyzed using IEDB MHC-1 binding prediction tool to predict cytotoxic T cell epitopes which interacted with different types of MHC Class I alleles in Man. Based on ANN with percentile rank ≤ 1 and ANN IC-50 ≤ 100. The top five were 110 YIMDNAEKL 118, 274 FYHRMYYPL 282, 278 MYYPLFSVF 286 and 231 YVVDNDRYV 239, 317 LLSGVFLAY 325 (Table 4 and Figure 2). Epitopes and their corresponding alleles were shown in Table 5.

Prediction of T helper cell epitopes and modelling

There were five T helper cell conserved epitopes resulted when applied FPV140 protein reference sequence to IEDB MHC-II binding prediction tool to interact with Man MHC II alleles based on nn-align with percentile rank ≤ 10 and nn IC50 ≤ 500, the top five were 110 YIMDNAEKL 118, 155 LQLVTHTKL163, 100 FIADHISLW 108, 136 FITNLDNIT 144, and 157 LVTHTKLLK 165 interacted with five epitopes (Table 6 and Figure 3).

There is overlapping in this epitope 110 **YIMDNAEKL** 118 between MHC-I epitopes and MHC-II epitopes (Table 6).

Molecular docking of B-F alleles and predicted CTL epitope

The five suggested CTL peptides that interacted with selected man's MHC-1 alleles: 110 YIMDNAEKL 118, 274 FYHRMYYPL 282, 278 MYYPLFSVF 286 and 231 YVVDNDRYV 239, 317 LLSGVFLAY 325 were used as ligands to detect their interaction with selected BF alleles / receptors (BF2*2101, BF2*0401) Figure 4 by docking Techniques using on-line software. Based on the binding energy in kcal/mol unit, the lowest binding energy (kcal/mol) was selected to obtain best binding and to predict real CTL epitopes as possible, (Figures 5a and 5b).

Discussion

Vaccination is a method to protect and minimize the possibility of infection. In the past there are many type of vaccines used, the most common one is a live attenuated vaccine though it provides the needed immunity but it may cause infection or allergy because it contains the necessary and much unnecessary proteins, in the other hand epitopes based vaccine is just include epitopes which responsible for inducing B and T cell mediated immunity. Nowadays it's used for many serious diseases such as HIV, Hepatitis B, cancer and for zoonotic viruses like Newcastle disease and avian influenza. In this study FPV 140 used as a target in the designing of peptide based vaccine against Fowlpox virus which is wide spread and had outbreaks in Brazil 1997 and China 2009 which led to severe economic plunge [2,5,19,44].

For good B cell epitope prediction the selected peptide should pass the threshold scores in Bepipred linear epitope prediction, Emini surface accessibility, Kolaskar and Tongaonkar antigenicity and Parker hydrophilicity prediction methods. Eight B cell epitopes were predicted by Bepipred linear epitope prediction. Seven epitopes (50 PPSPKP 55, 51 PSPKPL 56, 52 SPKPLP 57, 53 PKPLPK 58, 54 KPLPKS 59, 55 PLPKSK 60, 56 LPKSKQ 61) from 34 WSYKKGIKNGYDDYRDPPSPKPLPKSKQEPNADDKVGDIE 73 in addition to 18 RPSSTV 23 from 17 GRPSSTVV 24 succeed the Emini surface accessibility, Kolaskar and Tongaonkar antigenicity and Parker hydrophobicity prediction tools. Sometimes may no peptide pass specific test like in Zika virus study Badawi et al. has no peptide passed antigenicity test [44], or as in Newcastle study there was no conserved peptide passed the three test (surface accessibility, antigenicity and hydrophilicity) [49].

The B cell immunity stands for short time so that T cell immunity is required and important because it's long lasting and the CD4 and CD8 has main role in antiviral immunity. Therefore designing of peptide

No.	Start	End	Peptide	Length	Emini surface accessibility/ Threshold 1.000	Kolaskar and Tongaonkar antigenicity/ Threshold 1.026	Parker hydrophobicity prediction/ Threshold 1.000
1	1	6	**MAPGDK**	6	1.002	0.937	3.567
2	17	24	**GRPSSTVV**	8	0.48	1.064	2.85
3	34	73	**WSYKKGIKNGYDDYRDPPSPKPLPK-SKQEPNADDKVGDIE**	40	10.331	0.982	3.66
4	83	84	**GY**	2	1.002	0.937	3.567
5	116	118	**EKL**	3	1.273	1.01	1.433
6	128	132	**DNTIT**	5	1.008	0.922	3.88
7	207	213	**TNNKPSF**	7	1.989	0.937	3.471
8	238	238	**Y**	1	1.207	1.161	-1.9

Table 2: The predicted epitopes by Bepipred linear epitope prediction.

No.	Start	End	Peptide	Emini surface accessibility score/ threshold 1.000	antigenicity score/ threshold 1.026	hydrophobicity score/ threshold 1.000
1	18	23	**RPSSTV**	1.142	1.042	3.467
2	50	55	**PPSPKP**	3.004	1.033	3.433
3	51	56	**PSPKPL**	1.602	1.064	1.550
4	52	57	**SPKPLP**	1.602	1.064	1.550
5	53	58	**PKPLPK**	2.391	1.050	1.417
6	54	59	**KPLPKS**	2.072	1.042	2.150
7	55	60	**PLPKSK**	2.072	1.042	2.150
8	56	61	**LPKSKQ**	2.320	1.033	2.800

Table 3: Peptides predicted as epitopes (pass Emini surface accessibility, Kolaskar and Tongaonkar antigenicity and Parker hydrophobicity prediction tools).

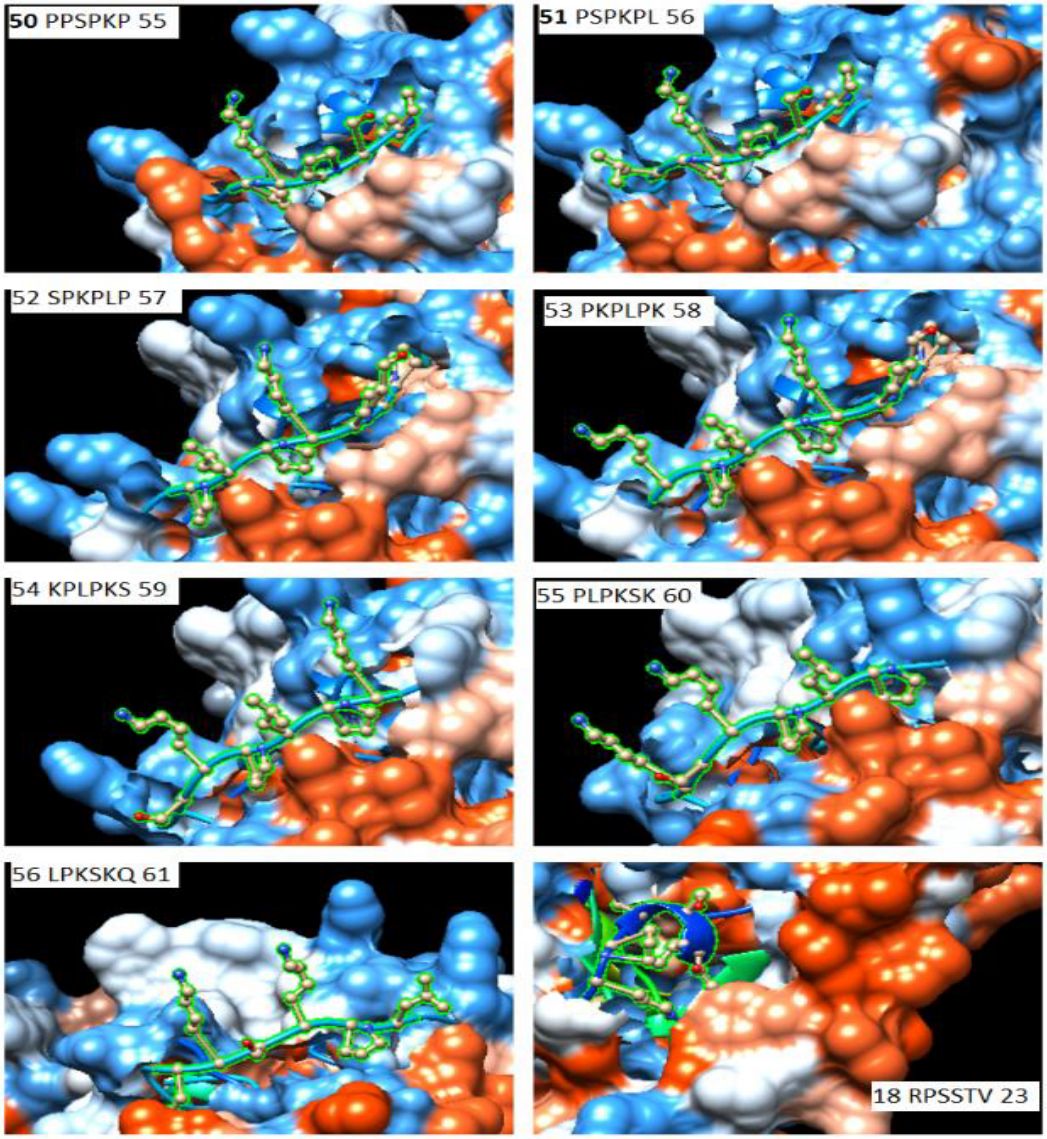


Figure 1: 3D structure of Predicted B cell epitopes of FPV140 protein in FPV virus illustrated by UCSF Chimera visualization tool.

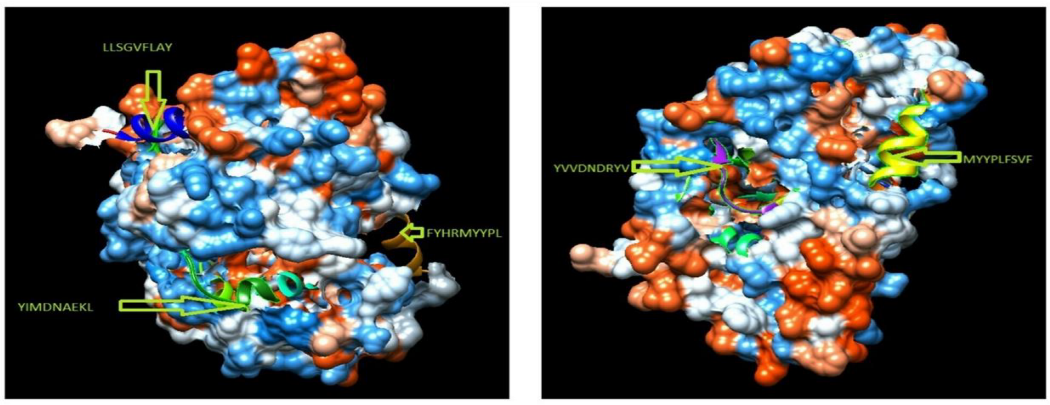


Figure 2: 3D structure of cytotoxic T cell top five epitopes.

Start	End	Peptide	Allele	Length	ic50	Percentile
8	16	**QIIFVITTI**	HLA-A*32:01	9	82	0.7
18	26	**RPSSTVVPF**	HLA-B*07:02	9	13	0.3
			HLA-B*35:01	9	9	0.4
			HLA-B*53:01	9	51	0.3
30	38	**EVSEWSYKK**	HLA-A*68:01	9	16	0.4
72	80	**IEYDEMVSV**	HLA-B*40:02	9	40	0.6
			HLA-C*12:03	9	19	0.8
77	85	**MVSVRDGYY**	HLA-A*29:02	9	17	0.4
			HLA-A*30:02	9	18	0.3
83	91	**GYYSDVCRL**	HLA-C*07:02	9	68	0.3
			HLA-C*14:02	9	4	0.2
99	107	**IFIADHISL**	HLA-C*14:02	9	25	0.9
100	108	**FIADHISLW**	HLA-A*26:01	9	63	0.3
101	109	**IADHISLWR**	HLA-C*05:01	9	50	0.7
110	118	***YIMDNAEKL**	HLA-A*02:01	9	20	0.8
			HLA-C*03:03	9	3	0.2
			HLA-C*07:01	9	83	0.8
			HLA-C*12:03	9	16	0.7
			HLA-C*14:02	9	10	0.4
			HLA-C*15:02	9	72	0.7
114	122	**NAEKLPNYV**	HLA-C*12:03	9	26	1
115	123	**AEKLPNYVV**	HLA-B*40:02	9	34	0.5
137	145	**ITNLDNITK**	HLA-A*11:01	9	36	0.8
154	162	**ILQLVTHTK**	HLA-A*03:01	9	92	0.7
			HLA-A*11:01	9	44	1
166	174	**DRNSQHLML**	HLA-C*06:02	9	79	0.4
			HLA-C*07:01	9	28	0.3
173	181	**MLLPDLEAF**	HLA-B*15:01	9	45	0.7
191	199	**AYIIRQEAV**	HLA-C*14:02	9	17	0.6
192	200	**YIIRQEAVR**	HLA-A*68:01	9	21	0.6
194	202	**IRQEAVRKL**	HLA-C*06:02	9	17	0.2
			HLA-C*07:01	9	14	0.2
197	205	**EAVRKLYSY**	HLA-A*26:01	9	40	0.3
205	213	**YFTNNKPSF**	HLA-C*07:02	9	82	0.3
			HLA-C*14:02	9	6	0.3
211	219	**PSFDISLEI**	HLA-C*15:02	9	78	0.8
220	228	**LRIENTLGI**	HLA-C*06:02	9	26	0.2
			HLA-C*07:01	9	23	0.3
224	232	**NTLGITRYV**	HLA-A*68:02	9	6	0.3
			HLA-C*15:02	9	53	0.5
230	238	**RYVVDNDRY**	HLA-A*30:02	9	94	0.8
231	239	***YVVDNDRYV**	HLA-A*02:06	9	15	1
			HLA-A*68:02	9	25	1
			HLA-C*07:01	9	94	0.8
			HLA-C*12:03	9	14	0.6
			HLA-C*15:02	9	71	0.7
232	240	**VVDNDRYVY**	HLA-A*01:01	9	80	0.3
			HLA-A*30:02	9	47	0.5
			HLA-C*05:01	9	7	0.3
237	245	**RYVYHDYKL**	HLA-A*23:01	9	78	0.5
241	249	**HDYKLANEF**	HLA-B*40:02	9	73	0.8
249	257	**FMKNKKNRL**	HLA-B*08:01	9	10	0.2
260	268	**KSRIDGWIM**	HLA-B*57:01	9	90	0.5
266	274	**WIMDNWPSF**	HLA-B*15:01	9	39	0.5
			HLA-B*35:01	9	17	0.6
267	275	***IMDNWPSFY**	HLA-A*01:01	9	7	0.2
			HLA-A*29:02	9	9	0.4
			HLA-A*30:02	9	18	0.3
			HLA-C*05:01	9	26	0.6
271	279	**WPSFYHRMY**	HLA-B*35:01	9	11	0.4
272	280	**PSFYHRMYY**	HLA-A*29:02	9	27	0.6
274	282	***FYHRMYYPL**	HLA-A*23:01	9	26	0.3
			HLA-A*24:02	9	19	0.2
			HLA-B*08:01	9	87	0.6
			HLA-B*39:01	9	18	0.3
			HLA-C*07:02	9	18	0.1
			HLA-C*14:02	9	3	0.1
275	283	**YHRMYYPLF**	HLA-C*07:01	9	71	0.7
			HLA-C*14:02	9	21	0.7
277	285	**RMYYPLFSV**	HLA-A*02:01	9	5	0.3
			HLA-A*02:06	9	7	0.6
			HLA-A*32:01	9	21	0.3
278	286	***MYYPLFSVF**	HLA-A*23:01	9	9	0.1
			HLA-A*24:02	9	27	0.3
			HLA-A*29:02	9	79	0.9
			HLA-B*15:01	9	65	0.9
			HLA-C*07:02	9	25	0.2
			HLA-C*14:02	9	3	0.1
281	289	**PLFSVFGKY**	HLA-A*29:02	9	55	0.8
			HLA-A*30:02	9	37	0.5
289	297	**YDITMMFLI**	HLA-B*40:02	9	73	0.8
291	299	**ITMMFLIAI**	HLA-A*32:01	9	19	0.3
292	300	**TMMFLIAIV**	HLA-A*02:01	9	8	0.4
293	301	**MMFLIAIVI**	HLA-A*32:01	9	9	0.2
			HLA-B*39:01	9	49	0.7
294	302	**MFLIAIVII**	HLA-A*23:01	9	85	0.5
295	303	**FLIAIVIII**	HLA-A*02:01	9	9	0.4
297	305	**IAIVIIIGL**	HLA-C*03:03	9	9	0.9
313	321	**KLLWLLSGV**	HLA-A*02:01	9	6	0.3
			HLA-A*02:06	9	4	0.3
314	322	**LLWLLSGVF**	HLA-B*15:01	9	28	0.3
316	324	**WLLSGVFLA**	HLA-A*02:01	9	5	0.3
			HLA-A*02:06	9	8	0.6
317	325	***LLSGVFLAY**	HLA-A*01:01	9	69	0.3
			HLA-A*03:01	9	78	0.6
			HLA-A*29:02	9	4	0.2
			HLA-A*30:02	9	71	0.7
			HLA-B*15:01	9	21	0.2

Table 4: The cytotoxic T cell epitopes and their corresponding alleles *Top five epitopes suggested for docking.

vaccine against T cell is more promising and effective. The T cell predicted epitopes is measured by binding affinity between the peptide and MHC alleles but unfortunately there is no database for chicken allele so the human allele is used as model due to similarity between human and chicken alleles (B-F and B-L alleles) [50,51] therefore HLA A, HLA B and HLA C is used for MHC I while HLA DR, HLA DQ and HLA DP is used for MHC II.

For CTL epitopes prediction ANN method was used with percentile rank ≤ 1 and IC-50 ≤ 100; fifty one conserved epitopes were predicted to interact with Man MHC-1 alleles, eighteen peptides interacted with 2-4 alleles, the top five epitopes 110 YIMDNAEKL 118, 274 FYHRMYYPL 282, 278 MYYPLFSVF 286 and 231 YVVDNDRYV 239, 317 LLSGVFLAY 325 interacted with six and five epitopes respectively (Figure 2).

T helper cell five conserved epitopes resulted when applied FPV140 protein reference sequence to IEDB MHC-II binding prediction tool to interact with Man MHC II alleles, based on nn-align with percentile rank ≤ 10 and IC50 ≤ 500, 110 YIMDNAEKL 118, 155 LQLVTHTKL 163 interacted with nine epitopes followed by 100 FIADHISLW108

Core Sequence	Start	End	Allele	Peptide Sequence	IC50	Rank
FIADHISLW	100	108	HLA-DRB1*03:01	TKIFIADHISLWRYI	16.7	0.92
				DTKIFIADHISLWRY	18	1.01
				KIFIADHISLWRYIM	28.5	1.67
				EDTKIFIADHISLWR	34	2.02
				IFIADHISLWRYIMD	60	3.32
				TEDTKIFIADHISLW	67.4	3.68
				FIADHISLWRYIMDN	236.8	8.92
			HLA-DRB1*04:01	TKIFIADHISLWRYI	20.7	1.03
				DTKIFIADHISLWRY	23.3	1.27
				EDTKIFIADHISLWR	29.4	1.84
				KIFIADHISLWRYIM	29.6	1.86
				TEDTKIFIADHISLW	42	3.05
				IFIADHISLWRYIMD	52.1	4.02
			HLA-DRB1*07:01	TEDTKIFIADHISLW	45.3	7.99
			HLA-DRB3*01:01	DTKIFIADHISLWRY	4.1	0.05
				TKIFIADHISLWRYI	4.1	0.05
				EDTKIFIADHISLWR	4.2	0.06
				TEDTKIFIADHISLW	4.3	0.07
				KIFIADHISLWRYIM	5	0.12
				IFIADHISLWRYIMD	6.7	0.25
				FIADHISLWRYIMDN	9.5	0.47
			HLA-DQA1*05:01/DQB1*02:01	EDTKIFIADHISLWR	331	7.47
				DTKIFIADHISLWRY	371.3	8.4
				TEDTKIFIADHISLW	414.9	9.36
			HLA-DPA1*01/DPB1*04:01	KIFIADHISLWRYIM	186.7	8.16
				TKIFIADHISLWRYI	216.5	8.98
			HLA-DPA1*01:03/DPB1*02:01	TKIFIADHISLWRYI	37.1	4.08
				KIFIADHISLWRYIM	40.7	4.39
				DTKIFIADHISLWRY	44.8	4.72
				EDTKIFIADHISLWR	56.4	5.62
				IFIADHISLWRYIMD	60.1	5.88
				TEDTKIFIADHISLW	64.4	6.17
				FIADHISLWRYIMDN	89.3	7.76
			HLA-DPA1*03:01/DPB1*04:02	TKIFIADHISLWRYI	18.9	2.14
				KIFIADHISLWRYIM	21.9	2.55
				DTKIFIADHISLWRY	23.1	2.72
				IFIADHISLWRYIMD	30.7	3.67
				EDTKIFIADHISLWR	37.6	4.45
				TEDTKIFIADHISLW	70.3	7.55
***YIMDNAEKL**	110	118	HLA-DRB1*01:01	LWRYIMDNAEKLPNY	10	5.19
			HLA-DRB1*03:01	LWRYIMDNAEKLPNY	30.2	1.77
				WRYIMDNAEKLPNYV	46.4	2.7
				SLWRYIMDNAEKLPN	60	3.32
				RYIMDNAEKLPNYVV	75.7	4.02
				ISLWRYIMDNAEKLP	114.3	5.52
				YIMDNAEKLPNYVVI	149.2	6.64
				HISLWRYIMDNAEKL	188.3	7.73
			HLA-DRB1*04:01	SLWRYIMDNAEKLPN	37.6	2.63
				LWRYIMDNAEKLPNY	39.3	2.79
				ISLWRYIMDNAEKLP	45.4	3.36
				HISLWRYIMDNAEKL	50	3.81
				WRYIMDNAEKLPNYV	81.3	6.58
			HLA-DRB1*07:01	HISLWRYIMDNAEKL	32.7	6.16
				ISLWRYIMDNAEKLP	35.5	6.56
				SLWRYIMDNAEKLPN	41.6	7.45

			HLA-DRB1*07:01	LWRYIMDNAEKLPNY	44.3	7.85
				LWRYIMDNAEKLPNY	38.8	2.3
				SLWRYIMDNAEKLPN	48.1	3.08
				ISLWRYIMDNAEKLP	56.6	3.76
				HISLWRYIMDNAEKL	64	4.31
				WRYIMDNAEKLPNYV	65.6	4.44
				RYIMDNAEKLPNYVV	102.1	7.06
			HLA-DRB1*13:02	LWRYIMDNAEKLPNY	20.2	1.45
				SLWRYIMDNAEKLPN	21.2	1.52
				ISLWRYIMDNAEKLP	22.6	1.62
				WRYIMDNAEKLPNYV	23.6	1.7
				HISLWRYIMDNAEKL	25.8	1.87
				RYIMDNAEKLPNYVV	37.3	2.67
				YIMDNAEKLPNYVVI	61.1	4.15
			HLA-DRB3*01:01	LWRYIMDNAEKLPNY	8	0.35
				SLWRYIMDNAEKLPN	8.6	0.4
				ISLWRYIMDNAEKLP	9.4	0.46
				WRYIMDNAEKLPNYV	10.4	0.55
				HISLWRYIMDNAEKL	10.5	0.56
				RYIMDNAEKLPNYVV	15.1	0.9
				YIMDNAEKLPNYVVI	25	1.55
			HLA-DRB5*01:01	HISLWRYIMDNAEKL	26.5	6.23
				LWRYIMDNAEKLPNY	27.1	6.34
				SLWRYIMDNAEKLPN	28.8	6.65
				ISLWRYIMDNAEKLP	30.8	7.01
				WRYIMDNAEKLPNYV	49.7	9.95
			HLA-DQA1*05:01/DQB1*02:01	HISLWRYIMDNAEKL	164.7	3.42
				ISLWRYIMDNAEKLP	180	3.81
				SLWRYIMDNAEKLPN	247.1	5.47
				LWRYIMDNAEKLPNY	311.1	7
				WRYIMDNAEKLPNYV	415	9.36
FITNLDNIT	136	144	HLA-DRB1*04:01	GEGFITNLDNITKVL	46.1	3.43
				TGEGFITNLDNITKV	65.7	5.25
				GFITNLDNITKVLND	72	5.8
				ITGEGFITNLDNITK	95.7	7.76
				FITNLDNITKVLNDN	119.1	9.58
			HLA-DRB1*04:04	ITGEGFITNLDNITK	50.1	5.71
			HLA-DRB1*08:02	EGFITNLDNITKVLN	247.2	5.68
				GEGFITNLDNITKVL	387.2	9.35
			HLA-DRB1*13:02	GEGFITNLDNITKVL	43.8	3.09
				EGFITNLDNITKVLN	48.5	3.38
				TGEGFITNLDNITKV	69.2	4.58
				GFITNLDNITKVLND	76.3	4.97
				FITNLDNITKVLNDN	117	6.79
				ITGEGFITNLDNITK	144	7.86
			HLA-DRB3*01:01	GEGFITNLDNITKVL	289.6	8.53
				TGEGFITNLDNITKV	290.8	8.55
				TITGEGFITNLDNIT	294.8	8.62
				ITGEGFITNLDNITK	314	8.93
LQLVTHTKL	155	163	HLA-DRB1*07:01	NNVDILQLVTHTKLL	3.9	0.25
				NVDILQLVTHTKLLK	4.1	0.29
				VDILQLVTHTKLLKD	4.7	0.43
				DNNVDILQLVTHTKL	4.8	0.45
				DILQLVTHTKLLKDR	5.7	0.66
				ILQLVTHTKLLKDRN	6.4	0.81
				LQLVTHTKLLKDRNS	8.9	1.38
			HLA-DRB1*09:01	VDILQLVTHTKLLKD	119.8	8.22
				NVDILQLVTHTKLLK	147.6	9.97

			HLA-DRB1*11:01	VDILQLVTHTKLLKD	25.7	4.73
				DILQLVTHTKLLKDR	29	5.34
				ILQLVTHTKLLKDRN	30.1	5.53
				NVDILQLVTHTKLLK	30.7	5.64
				LQLVTHTKLLKDRNS	50.7	8.6
			HLA-DRB1*15:01	NVDILQLVTHTKLLK	77.5	7.9
				NNVDILQLVTHTKLL	87.8	8.85
				VDILQLVTHTKLLKD	95.5	9.54
			HLA-DRB4*01:01	VDILQLVTHTKLLKD	32.7	2.16
				DILQLVTHTKLLKDR	33.2	2.21
				NNVDILQLVTHTKLL	34.2	2.3
				NVDILQLVTHTKLLK	34.2	2.3
				ILQLVTHTKLLKDRN	34.4	2.31
				DNNVDILQLVTHTKL	39.2	2.74
				LQLVTHTKLLKDRNS	51.1	3.81
			HLA-DPA1*01/DPB1*04:01	ILQLVTHTKLLKDRN	138	6.65
				DILQLVTHTKLLKDR	139	6.68
				VDILQLVTHTKLLKD	156.5	7.25
				NVDILQLVTHTKLLK	243.6	9.66
			HLA-DPA1*01:03/DPB1*02:01	DILQLVTHTKLLKDR	92.8	7.95
				VDILQLVTHTKLLKD	93.2	7.97
				ILQLVTHTKLLKDRN	97.6	8.23
			HLA-DPA1*02:01/DPB1*01:01	VDILQLVTHTKLLKD	32.6	3.18
				DILQLVTHTKLLKDR	33.4	3.28
				ILQLVTHTKLLKDRN	35.4	3.53
				NVDILQLVTHTKLLK	41	4.22
				LQLVTHTKLLKDRNS	54.9	5.85
				NNVDILQLVTHTKLL	55.6	5.92
				DNNVDILQLVTHTKL	90.6	9.55
			HLA-DPA1*02:01/DPB1*05:01	VDILQLVTHTKLLKD	192.2	4.24
LVTHTKLLK	157	165	HLA-DRB1*03:01	ILQLVTHTKLLKDRN	87.1	4.48
				DILQLVTHTKLLKDR	90.2	4.62
				VDILQLVTHTKLLKD	104.3	5.17
				NVDILQLVTHTKLLK	127.5	5.94
				LQLVTHTKLLKDRNS	207.5	8.21
			HLA-DRB5*01:01	ILQLVTHTKLLKDRN	36.1	7.93
			HLA-DPA1*01/DPB1*04:01	LQLVTHTKLLKDRNS	165	7.51
			HLA-DPA1*02:01/DPB1*05:01	ILQLVTHTKLLKDRN	160.2	3.48
				DILQLVTHTKLLKDR	178.3	3.91
			HLA-DPA1*03:01/DPB1*04:02	ILQLVTHTKLLKDRN	14.3	1.48
				DILQLVTHTKLLKDR	16.6	1.81
				LQLVTHTKLLKDRNS	17.3	1.91
				VDILQLVTHTKLLKD	21.9	2.55
				NVDILQLVTHTKLLK	31.5	3.76
				QLVTHTKLLKDRNSQ	43.6	5.11
				LVTHTKLLKDRNSQH	88.2	8.89

Table 5: Top T helper cell epitopes and interaction with MHC-II alleles.

Peptide	Start	End	BF2*2101 binding energy (kcal/mol)	BF2*0401 binding energy (kcal/mol)
YIMDNAEKL	110	118	-38.57	-25.85
FYHRMYYPL	274	282	-52.08	-49.43
MYYPLFSVF	278	286	-62.58	-*
YVVDNDRYV	231	239	-37.57	-39.41
LLSGVFLAY	317	325	-63.79	-68.52

Table 6: the docking energy Kcal/mol of BF alleles and CTL epitopes *not bind in ideal way with this receptor.

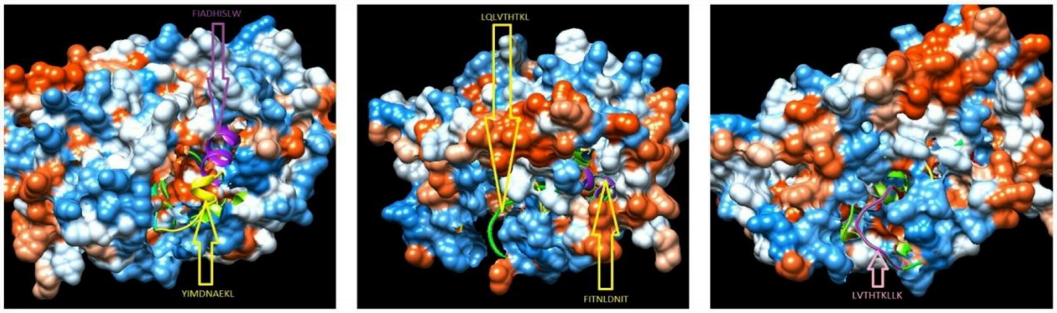


Figure 3: Top five T helper cell epitopes interacted with MHC-II alleles.

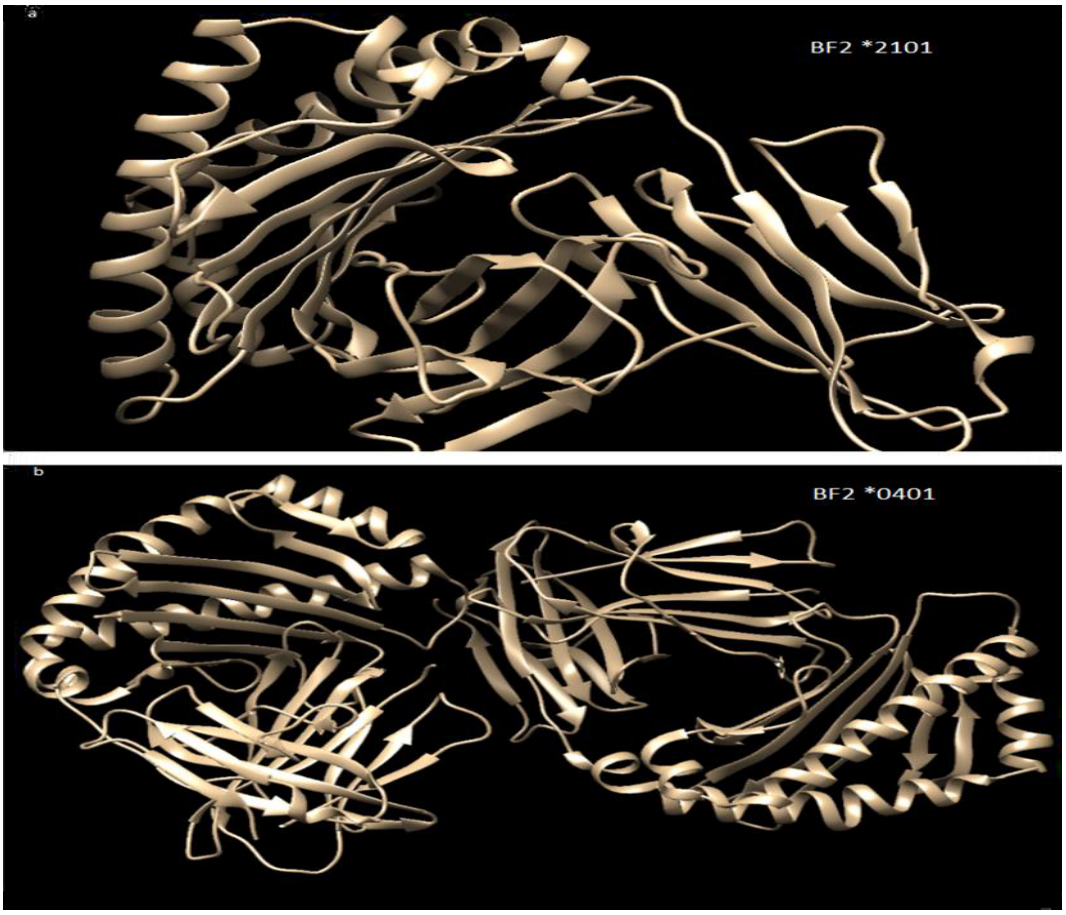
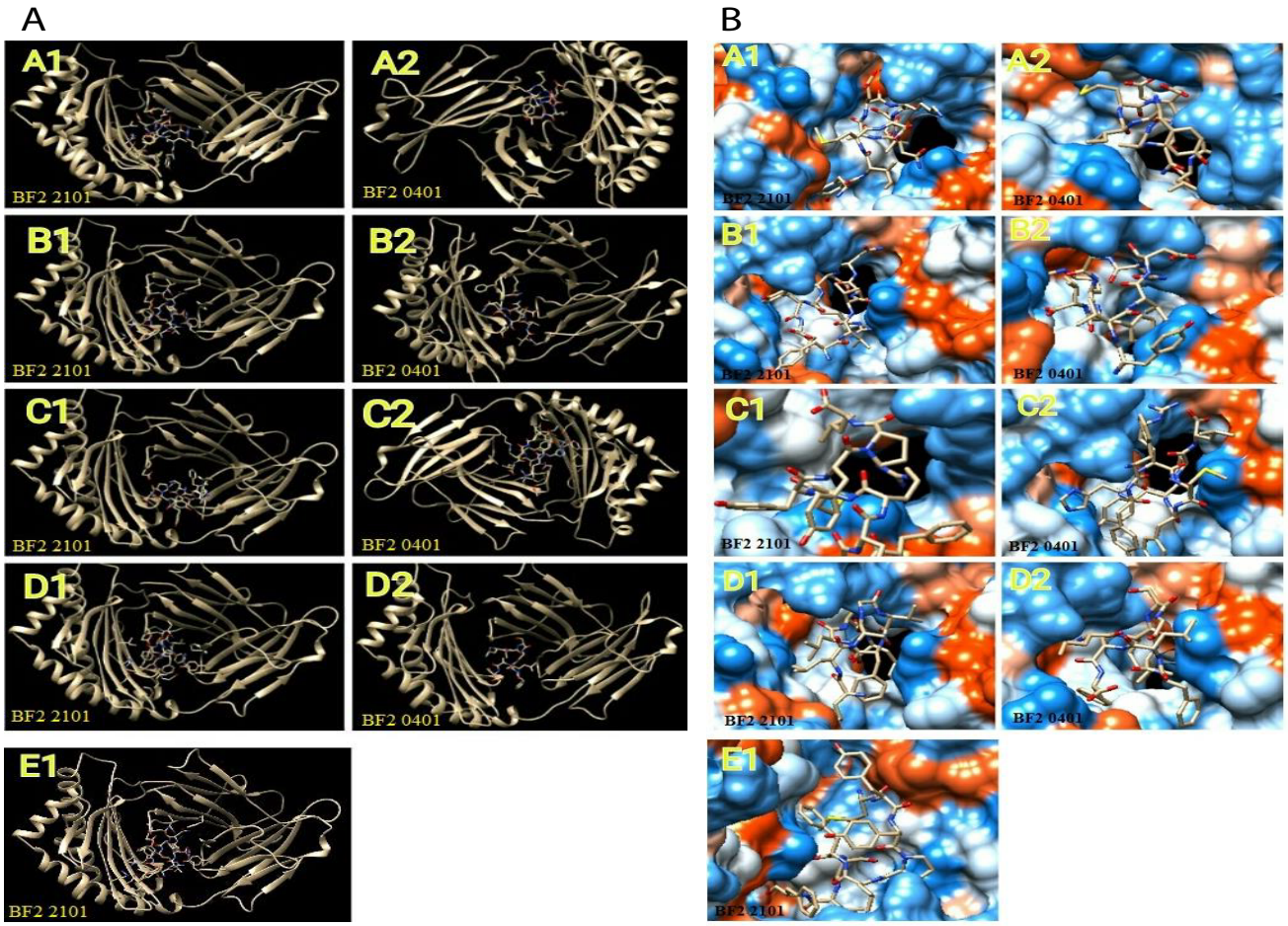


Figure 4: BF alleles (BF2*2101, BF2*0401).

with eight epitopes and lastly 136 FITNLDNIT 144, 157 LVTHTKLLK 165 with five epitopes (Table 6 and Figure 3).

There is overlapping in 110 YIMDNAEKL 118 epitope between MHC-I epitopes and MHC-II epitopes (Table 6). Its interacted with (HLA-A*02:01, HLA-C*03:03, HLA-C*07:01, HLA-C*12:03, HLA-C*14:02, HLA-C*15:02)MHC-I alleles and (HLA-DRB1*01:01, HLA-DRB1*03:01, HLA-DRB1*04:01, HLA-DRB1*07:01, HLA-DRB1*07:01, HLA-DRB1*13:02, HLA-DRB3*01:01, HLA-DRB5*01:01, HLA-DQA1*05:01/DQB1*02:01)MHC-II alleles.

The CTL epitopes (110 YIMDNAEKL 118, 274 FYHRMYYPL 282, 278 MYYPLFSVF 286 231 YVVDNDRYV 239 and 317 LLSGVFLAY 325) docked and interacted with BF2*2101, BF2*0401 to detect the presence of real CTL epitopes, theselection of those alleles depend on Kokh et al. study who reported the presence of the first structures of an MHC molecule (BF2*2101) in chicken MHC haplotype B21, not in mammals, Zhang J et al. study who reported the crystal structure of BF2*0401 from the B4 haplotype, Osman et al. used those alleles for docking [41,42,47]. The lowest binding energy (k cal/mol) for (BF2*2101) (BF2*0401) alleles shown by 317 LLSGVFLAY 325 followed by 278 MYYPLFSVF 286 which is not bind with BF2*0401 in ideal way, 274 FYHRMYYPL 282, 231



Figures 5a and b: the interaction between epitopes and receptors (BF2*2101, BF2*0401) using UCSF-Chimera visualization tool after online docking. **A:** YIMDNAEKL, **B:** YVVDNDRYV **C:** FYHRMYYPL, **D:** LLSGVFLAY, **E:** MYYPLFSVF This epitope (MYYPLFSVF) not interacted with receptor BF2*0401 in ideal way.

YVVDNDRYV 239 and 110 YIMDNAEKL 118. Those docked epitopes suggested to be peptide vaccine.

Concisely the five docked epitopes suggested to be peptide vaccine especially 110 YIMDNAEKL 118 it overlapped between CTL epitopes and T helper cell according to these result it will give good vaccine if applied *in vivo* and *in vitro* and it will short the time and cost for vaccine production but also we recommend more studies for FPV peptide vaccine due to small sample size in this study and the importance of this vaccine for poultry population.

Conclusion

In this study we tried out to design epitope based vaccine against FPV, which could be test for efficacy in activation of humoral and cell mediated immunity. This study gave a computational data which help in vaccine identification and designing with safety and less cost, thus led to prevention of infection through poultry population. Our result based on sequence analysis and *in silico* prediction though *in vitro* and *in vivo* studies required as long with *in silico* study to prove the effectiveness of vaccine.

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