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Multi Epitope Based Peptide Vaccine against Marek's Disease Virus Serotype 1 Glycoprotein H and B

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Abstract **Background:** Marek's disease (MD) is a highly contagious disease of chickens caused by Marek's disease virus (MDV). It causes economic losses in poultry industry estimated to be more than 1 billion per year. The aim of this study was to design a peptide vaccine against Marek's disease virus serotype 1 (MDV-1) by targeting the Glycoproteins H and B as an immunogens to stimulate protective immune response. A total of 43 Glycoprotein H and 33 glycoprotein B of Gallid alphaherpesvirus 2 (MDV-1) were retrieved from the National Center for Biotechnology Information database (NCBI) in the 13th of October 2017. Several tests at Immune Epitope Database (IEDB) were used to detect the highly conserved immunogenic epitopes that elicit B and T cells and could be used as efficient vaccine candidates. In our results three epitopes from glycoprotein H namely; $_{91}$-**FYKRPVSKLL**-$_{100}$, $_{255}$-**LKPYEPVDKF**-$_{264}$, and $_{684}$-**PRPL**-$_{687}$ and three epitopes of glycoprotein B; $_{162}$- **EKQV**-$_{165}$, $_{234}$-**YGLSPPE**-$_{240}$, and $_{363}$-**YNDSHVK**-$_{369}$ were fulfilled the criteria of surface accessibility, antigenicity for becoming the most probable B cell epitope. While Four epitopes of glycoprotein H; $_{425}$-**YVLRSAYAF**-$_{433}$, $_{175}$-**LTSELTGTY**-$_{183}$, $_{476}$-**LYYAFASIF**-$_{484}$, and $_{367}$-**MITETLSTF**-$_{375}$ were addressed as potentially promising epitopes as they bound the highest number of both MHC-I and MHC-II alleles with a high binding affinity to chickens MHC-I molecule (BF2*2101) haplotype in the structural level. Also two epitopes of glycoprotein B; $_{598}$-**FLFGSGYAL**-$_{606}$, $_{727}$-**FMSNPFGAL**-$_{735}$ were bound with the highest number of both MHC-I and MHC-II with high binding affinity. Taken together Marek's disease is a significant disease of poultry. We addressed epitopes from glycoprotein H and B that could act as candidates' vaccine. To our knowledge there is no *in silico* epitope based vaccine for Marek's disease virus serotype 1 (MDV-1). An in vitro and in vivo application is required to prove the efficacy of the predicted epitopes as peptide vaccine.

Keywords: *Marek's disease (MD), Vaccination, Immunoinformatics, Glycoprotein H, Glycoprotein B*

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1. Introduction

Marek's disease (MD) is a highly contagious disease of chickens caused by Marek's disease virus (MDV) leads to the formation of T-cell tumors in various body tissues and neurological manifestations [1-5]. The disease transmitted by inhalation [6] and classified as a B-list infectious disease in Office International des Epizooties (OIE) with important economic losses in poultry industry estimated at more than 1 billion per year [7,8,9]. The morbidity and mortality rate depending on host genetic susceptibility and virulence of the MDV strain. The Mortality rates were commonly 10% to 30% and on incident could reach 60% to 80% [10,11,12]. Marek's disease (MD) is widespread in the worldwide. Recently, OIE estimates that about half of the world countries have reported cases of MDV infection such as the United States, the central Ethiopia, Saudi Arabia, Egypt, India [13-19], and China is an endemic area with outbreaks reported [20,21].

Marek's disease virus (MDV) is double-standard DNA that belongs to the *Herpesviridae* family, subfamily *Alphaherpesvirinae* in the genus *Mardivirus*. It has three serotypes different in their virulence in chicken or their susceptibility to induce T-cell lymphomas. These serotypes are Marek's disease virus serotype 1 (MDV-1) (Gallid herpesvirus 2), serotype 2 (Gallid herpesvirus 3), and serotype 3 (herpesvirus of turkeys) (HVT or MDV3) [22,23]. Among these serotypes, serotype 1 MDVs are oncogenic [24,25,26,27], while other serotypes are non-oncogenic chicken viruses [28,29,30]. MDV-1 genome is 175 to 180 kb, depending on the strain and is predicted to encode 103 glycoproteins [31,32]. Glycoproteins are virion

surface components and represent potent immunogens. Among them are glycoprotein B, C, D and H [33]. Glycoproteins H (gH) and B (gB) were the main target of immune response to the virus [4,34,35,36].

The MD is still good controlled by vaccinations as the first successful live vaccine to control MD is herpesvirus of turkey (HVT) since the 1970s [37]. HVT is a highly cell-associated virus but vaccine preparations require special handling and storage [38]. MDV strain required the introduction of new generations of MD vaccine such as CVI988 (Rispens) vaccine, a naturally attenuated MDV-1 to induce protection against very virulent plus MDV strains [39,40]. But these vaccines prevented the tumor development but not the MDV infection [41]. In spite of that, the recombinant DNA vaccines overcome these problems associated with MD vaccine [42,43] but it provided partial protection against MDV [38,44]. Therefore, it is important to consider alternative approaches for (MDV-1) vaccine development.

Peptide –based vaccine is a one of immunoinformatics applications. It is based on identification and chemical synthesis of B-cell epitopes which mainly induce antibody production and the T-cell epitopes that induce cellular response and cytokine secretion as cytotoxic T-cells [45-49]. Peptides have become more desirable vaccine candidates owing to their relatively easy production and construction, chemical stability, and absence of infectious potential, which lessens the time and reduce cost [45-49]. Moreover these approaches serve as therapeutic and vaccine candidates for many infectious diseases [50-54].

Therefore aim of this study was to design a peptide vaccine against Marek's disease virus serotype 1 (MDV-1) using immunoinformatics approach. Glycoprotein H and B were used as an immunogens to stimulate protective immune response.

2. Materials and Methods

To identify the best likely B- and T-cell peptides which could be used to design an effective vaccine, different methods were taken into attention in this study and an outline of the methodology was shown in Figure 1.

2.1. Protein Sequence Retrieval

A total of 43 Protein sequences of glycoprotein H and 33 of glycoprotein B of Gallid alphaherpesvirus (MDV-1) were retrieved from the National Center for Biotechnology Information database (NCBI) in the 13th of October 2017. The retrieved sequences and their accession numbers as well as the collection area were listed in Table 1.

2.2. Conserved Regions Determination

Retrieval sequences of Glycoprotein H and Glycoprotein B of MDV-1 were aligned to obtain the conserved regions using Multiple Sequence Alignment (MSA) by Clustal-W as applied in the Bio-Edit program (version 7.2.5.0) [55]. Which were considered as candidate epitopes were further analyzed by different prediction tools from Immune Epitope Database IEDB analysis resource (http: //www.iedb.org/)

2.3. B-cell Epitope Prediction

B-cell epitope is defined as part of antigen that is recognized by B-cell receptor to elicit antibody in humeral response [56]. The epitopes of a native protein must be accessible at the antigen surface and possess antigenic reactivity with antibodies. To predict these physicochemical and structural parameters are usually calculated based on propensity scales for each of the 20 amino acids [57,58]. The most epitope prediction tools available in immune epitope database IEDB. The reference sequences of glycoprotein H and glycoprotein B of MDV-1 were subjected to different B cell tools from immune epitope database (http://tools. iedb.org/bcell/) to predict linear B-cell epitopes using BepiPred linear epitope prediction, surface accessibility by Emini surface accessibility prediction tool, and antigenic reactivity using Kolaskar and Tongaonkar antigenicity tool.

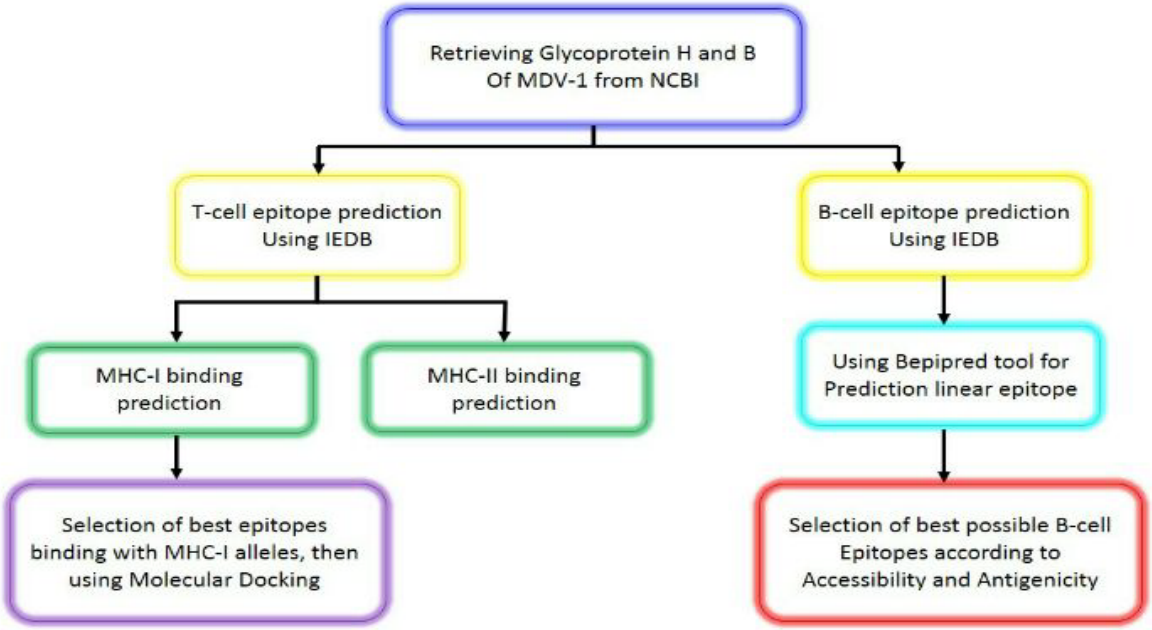

Figure 1. Flowchart displaying the protocols employed to predict B cell and T cell epitopes

Table 1. Glycoprotein H and glycoprotein B of MDV-1 retrieved strains, their accession numbers and their area of collection.

Glycoprotein H			Glycoprotein B		
Accession No	Year	Country	Accession No	Year	Country
YP_001033950**	2000	USA	YP_001033956**	2007	USA
AAM97722*	2002	USA	AAB02796*	1995	Australia
AAM97721*	2002	USA	AAM97702*	2002	USA
AAM97720*	2002	USA	AAM97701*	2002	USA
AAM97719*	2002	USA	AAM97700*	2002	USA
AAM97718*	2002	USA	AAM97699*	2002	USA
AEO45524	1995	China	AAM97698*	2002	USA
AEO45523	2005	China	AAF66762*	2004	USA
AEO45522	2005	China	AAS01668*	2003	USA
AEO45521	2005	China	AFM75523*	2012	USA
AEO45520	2008	China	AFM75345*	2012	USA
AEO45519	2008	China	AFM75164*	2012	USA
AEO45518	2008	China	AFM74977*	2012	USA
AEO45517	2007	China	AFM74787*	2012	USA
AEO45516	2007	China	AFM74600*	2012	USA
AEO45515	2007	China	ARE59074	2011	China
AEO45514	1995	China	AQN78163	2014	China
AEO45513*	2010	China	AQN77991	2014	China
AEO45512	2004	China	AQN77818	2014	China
AAF66757*	2004	USA	AQN77640	2012	China
AAS01661*	2003	USA	AQN77469	2011	China
AAP13936*	1993	U.K	AQN77293	2011	China
AFM75515*	2012	USA	AQN77119	1974	China
AFM75337*	2012	USA	AFX97943	2001	China
AFM75156*	2012	USA	AEZ51685	2007	China
AFM74969*	2012	USA	ACF94954*	2008	USA
AFM74779*	2012	USA	ABR13109*	2007	USA
AFM74592*	2012	USA	BAA02866***		
ABR13101*	2007	USA	AUB51196	2010	Poland
AFX97935	2001	China	AUB51111	2010	Hungary
ARE59066	2011	China	AUB51026	1992	Israel
AQN78155	2014	China	AUB50941	2000	Hungary
AQN77983	2014	China	AAG14220*	2000	USA
AQN77810	2014	China			
AQN77632	2012	China			
AQN77461	2011	China			
AQN77285	2011	China			
AQN77111	1974	China			
AEZ51677	2007	China			
ACF94933*	2008	USA			
ALX81087*	2015	USA			
ALX80969*	2015	USA			
AEV55001	1986	China			

*specific year of collection are not available, the written year refer to the year of submission in the NCBI database.
** Reference sequence.
***no information available.

2.4. T-cell Epitope Prediction

A functional T-cell response requires MHC–peptide binding and a suitable interaction of the MHC–peptide ligand with a specific T-cell receptor (TR) [45]. Therefore this requires the prediction of peptides bind to MHC class I &II.

2.4.1. Major Histocompatibility Complex (MHC) Class I Binding Predictions

For prediction of peptides bind to MHC class I; the reference sequence of Glycoprotein H and Glycoprotein B of MDV-1 were submitted in MHC-I Binding prediction tool (http: //tools.iedb.org/mhci/) in IEDB. Prediction method of Artificial Neural Network (ANN) [59] was used to calculate IC50 values of peptide binding to MHC1 molecules. Analysis was done using human leucocyte antigen (HLA) alleles [60]. For all the alleles, peptide length was set to 9 amino acids prior to the prediction [61]. The alleles having binding affinity IC_{50} less than 100 nm were suggested to be promising candidate epitopes.

2.4.2. Major Histocompatibility Complex (MHC) Class II Binding Predictions

For prediction of peptides bind to MHC class II; the reference sequence of Glycoprotein H Glycoprotein B of

MDV-1 were assessed by the IEDB MHC-II prediction tool at (http: //tools.iedb.org/mhcii/). For the screening of promising epitopes, human allele reference set (HLA DR, DQ, DP) were used [62,63]. NN-align prediction method [64] in IEDB was used with (IC_{50}) 0f 1000 nm. Peptides less or equal to the (IC_{50}) value were chosen for further consideration.

2.5. Homology Modeling

For creation of the 3D structure of the reference sequence of Glycoprotein H (YP_001033950.1) and glycoprotein B (YP_001033956) using Raptor X protein structure prediction server available at (http: // raptorx.uchicago.edu/Structure Prediction/predict/). Chimera 1.8 [65] was used to visualize the selected epitopes belonging to the B cell, MHC-I, and MHC-II.

2.6. Molecular Docking of the Proposed Epitopes with MHC Class 1 Alleles

The MHC class I of Chickens BF2*2101 haplotype (CAK54661.1) was retrieved in FASTA format in the 15th of October 2017 from the National Center for Biotechnology Information (NCBI) and uploaded to the Raptor X (http: //raptorx.uchicago.edu/StructurePrediction/predict/) to obtain the 3D structure. Four peptides of glycoprotein H and two peptides of glycoprotein B are bound the largest number of alleles were selected to predict 3D peptide structure using Pep fold available at the mobyle web server [66]. The proposed models for each of the selected MHC class I binding peptides were docked with the MHC class I protein (BF2*2101) using Patch dock server [67] and visualized using Chimera 1.8.

3. Results

3.1. Conserved Regions Determination

Figure 2 showed the conserved regions in the retrieval sequences of Glycoprotein H and Glycoprotein B of MDV-1. Moreover the thresholds for B- Cells for the linear epitopes, surface accessibility and antigenicity were shown in Figure 3

3.2. B-cell Epitope Prediction

The reference sequence of glycoprotein H and glycoprotein B of MDV-1 were subjected to different tools in B-cell epitope prediction at IEDB. Several epitopes were predicted to interact against B cells for both glycoproteins H and B. Glycoprotein H epitopes were shown in Table 2 while the best three epitopes of glycoprotein H were shown in Table 3 with their scores. Table 4 showed the total epitopes that obtained from glycoprotein B and Table 5 demonstrated the best three epitopes from Glycoprotein B with their scores. These selected epitopes fulfilled the criteria of surface accessibility, antigenicity. Moreover the 3D structures of the selected epitopes from glycoprotein H and B were shown in Figure 4.

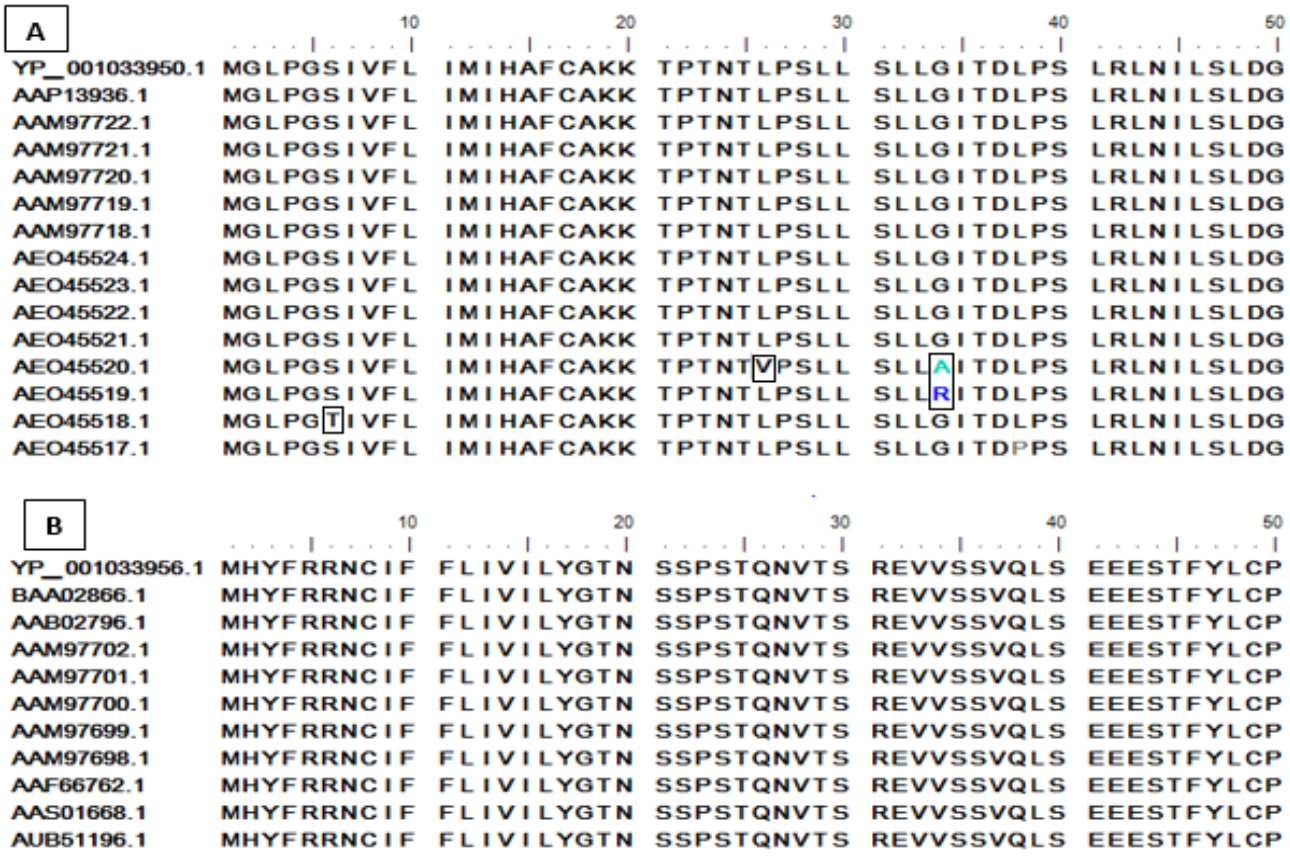

Figure 2. Multiple Sequences Alignment (MSA), **A**; for glycoprotein H and **B**; for glycoprotein B. The boxes within the sequences showed position of mutations among the retrieved strains

Table 2. B- Cells conserved peptides of glycoprotein H with their surface accessibility score and antigenicity score

Peptide	Start	End	Length	Emini score *a	Antigenicity score *b
MGLP	1	4	4	0.52	1.004
AKKTPTNT	18	25	8	5.198	0.936
LDGSANNQGSWVRDNT	48	63	16	1.756	0.951
IGASSPANG	68	76	9	0.431	0.988
FYKRPVSKLL	91	100	10	1.226	1.094
MTAM	120	123	4	0.594	0.906
PYRRNVQ	125	131	7	4.133	1.021
PSDRSGLKLDDKDD	133	146	14	8.183	0.966
QPTGTNPPTELKNLKPIDVVNP	148	169	22	3.947	1.012
KNLKPIDVVNP*	159	169	11	1.144	1.052
ELTGT	178	182	5	0.982	0.959
FGSDGAE	209	215	7	0.714	0.947
SMKSAPPVEL	230	239	10	1.003	1.046
LKPYEPVDKFTR	255	266	12	4.461	1.031
LKPYEPVDKF*	255	264	10	2.532	1.059
HMNASDMEI	277	285	9	0.81	0.942
ESVEESSNY	293	301	9	3.221	0.99
FQIAQT	303	308	6	0.71	1.041
TPIS	318	321	4	0.872	1.034
RNAFQS	345	350	6	1.71	0.972
IIKTEANIK	358	366	9	0.771	0.991
LHSNPGTY	377	384	8	1.44	1.019
LHSNP*	377	381	5	1.248	1.041
LRNENAD	392	398	7	2.625	0.922
INASKNTAS	409	417	9	1.321	0.966
FNISQESGNLGEHVS	433	447	15	0.545	1.002
ESVRDTIAWNTS	460	471	12	1.294	0.975
FQRPPNEWDASRTAR	484	498	15	8.847	0.953
VKNSPV	529	534	6	0.982	1.091
KYLEND	572	577	6	3.215	0.972
KYLE*	572	575	4	1.863	1.048
RGIDAEEELETK	579	590	12	3.372	0.944
VVTCSDA	613	619	7	0.207	1.147
GLGSYV	634	639	6	0.337	1.092
VDGVDVN	653	659	7	0.39	1.076
PCTTT	670	674	5	0.831	1.041
PRPL	684	687	4	1.607	1.063
SDCPY	689	693	5	0.97	1.103
STNGNLR	702	708	7	1.72	0.924
SQDLQRELIAGGNSSIRYF	715	733	19	0.814	1.009
ATATAGAS	733	780	8	0.495	0.995
PTIAQI	735	740	6	0.513	1.059

* Peptide after being shortened. *a threshold 1.000, *b threshold 1.041.

Table 3. The best three B- Cell epitopes of glycoprotein H that gave score above threshold in Emini & Antigenicity

Peptide	Start	End	Length	Emini	Antigenicity
FYKRPVSKLL	91	100	10	1.226	1.094
LKPYEPVDKF	255	264	10	2.532	1.059
PRPL	684	687	4	1.607	1.063

Table 4. B- Cells conserved peptides of glycoprotein B with their surface accessibility score and antigenicity score.

Peptide	Start	End	Length	Emini score *a	Antigenicity score *b
TNSSPSTQNVTSREVV	19	34	16	1.118	1.017
QLSEEESTFYLCPPPVGSTVIRLEPPRKCPEPRKATEWGE	38	77	40	0.464	1.028
LEPPRKC	60	66	7	1.087	1.063
ISPY	87	90	4	0.778	1.097
TTTWTGTTYRQITNRYTDRTPVSIE	104	128	25	3.768	0.966
RTPV*	122	125	4	1.109	1.057
DLIDGKGRCSS	132	142	11	0.292	1.011
AFDRDAGEKQVLL	155	167	13	0.396	1.029
EKQV*	162	165	4	1.522	1.045
SKFNTPESRAWHTTN	170	184	15	2.871	0.945
TYTVWGSPWIYRTGTS	186	201	16	0.392	0.999
DARSV	210	214	5	0.86	1.04
GDIANI	225	230	6	0.264	0.981
FYGLSPPEA	233	241	9	0.54	1.048
YGLSPPE*	234	240	7	1.076	1.039
AEPMGYPQDNFKQ	243	255	13	2.449	0.969
DKRRKASLP	265	273	9	3.845	0.985
RKASLP*	268	273	6	1.355	1.032
GWDWAPKTTRV	287	297	11	0.847	0.969
ISNTTEFDP	330	338	9	1.059	0.959
KREAEAA	348	354	7	1.836	0.957
TKYNDSHVK	361	369	9	2.858	1.008
YNDSHVK*	363	369	7	1.727	1.033
RDNRTDE	404	410	7	6.519	0.859
KNATS	424	428	5	0.938	0.938
DIRNAPNRKITLD	435	447	13	1.383	0.965
TTAIKSTSS	449	457	9	0.891	0.99
IKINPSATASATLG	500	513	14	0.159	1.017
CTAIDAESVTL	530	540	11	0.093	1.079
NTCY	551	554	4	0.667	1.065
GENQGNIQGQLGENNELL	564	581	18	0.487	0.95
EAVEPCSAN	585	593	9	0.353	1.053
KEELR	648	652	5	2.536	0.951
VARR	662	665	4	0.984	1.048
GQVGQA	700	705	6	0.441	1.037
KLKSNPMKALYP	757	768	12	1.61	1.021
VLKAQATRELHGEESDDLERTSIDERKLEE	773	802	30	4.249	0.984
LKAQATREL*	774	782	9	1.023	1.023
AEERHEKKLRR	815	825	11	8.67	0.95
RRGTTAV	827	833	7	0.899	0.984

* Peptide after being shortened. *a threshold 1.000, *b threshold 1.022.

Table 5. The best three B- Cell epitopes from glycoprotein B that gave score above threshold in Emini & Antigenicity

Peptide	Start	End	Length	Emini Score	Antigenicity score
EKQV	162	165	4	1.522	1.045
YGLSPPE	234	240	7	1.076	1.039
YNDSHVK	363	369	7	1.727	1.033

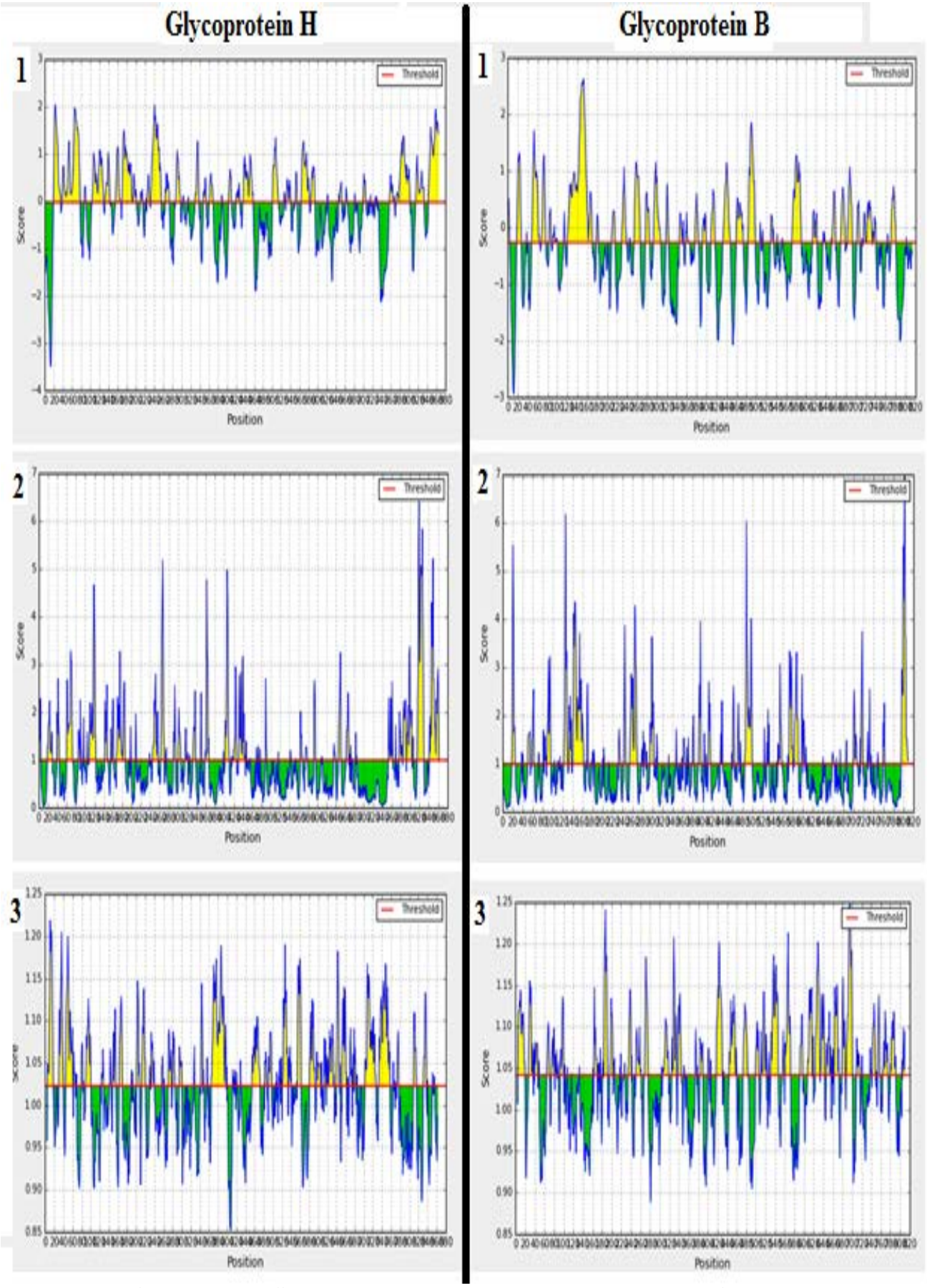

Figure 3. Prediction of B-cell epitopes by different IEDB scales (**1-** Bepipred linear epitope prediction, **2-** Emini surface accessibility, **3-** Kolaskar and Tongaonkar antigenicity prediction). Regions above threshold (red line) are proposed to be a part of B cell epitope while regions below the threshold (red line) are not

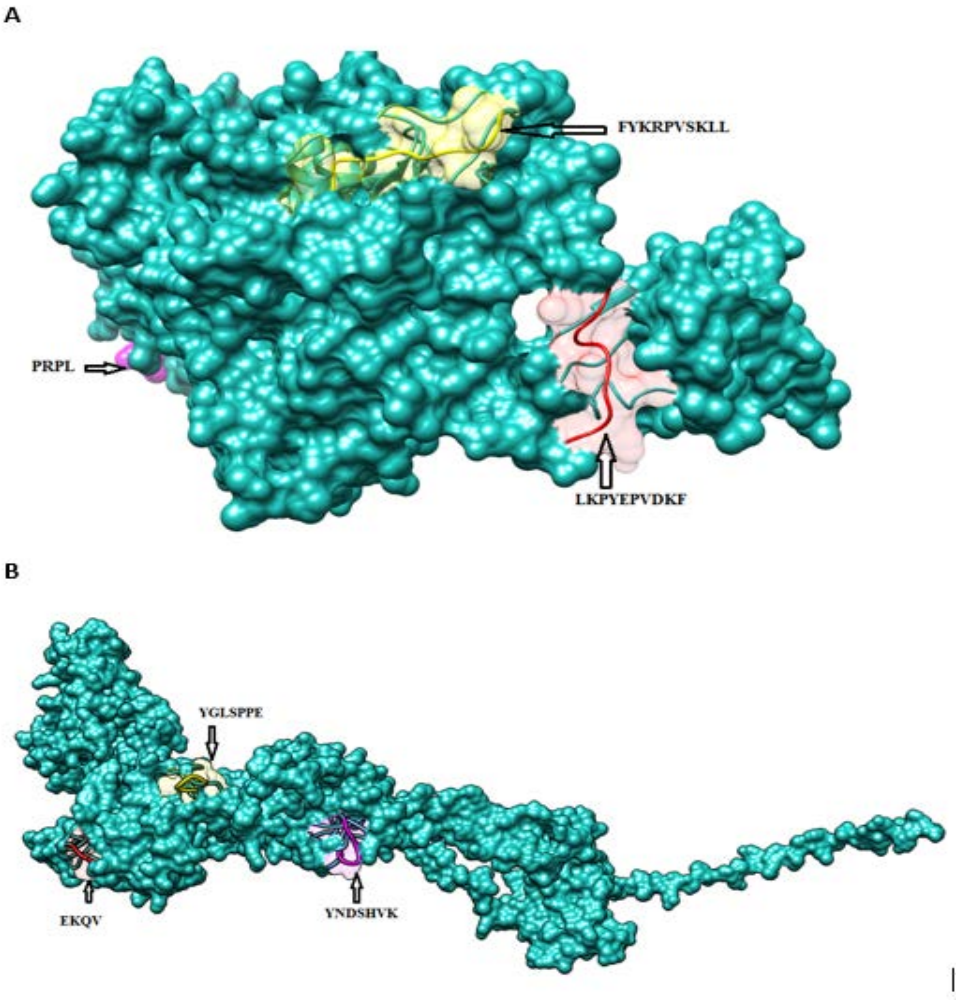

Figure 4. Structural position of the most promising conserved B cell epitopes; **A**: for glycoprotein H and **B**: for glycoprotein B of MDV-1

3.3. T-cell Epitope Prediction

3.3.1. MHC Class I Binding Predictions

MHC class-I of various HLA alleles with interaction of T-cell epitopes of glycoprotein H and glycoprotein B were predicted. Successful candidate's epitopes had a half maximal inhibitory concentration (IC50) < 100 nM, were listed in Table 6 and Table 7. The 3D structure of these epitopes was demonstrated in Figure 5.

Table 6. The best epitopes of glycoprotein H that had binding affinity with the human MHC class I alleles

Peptide	Start	End	Allele	ic50	Percentile
YVLRSAYAF	425	433	HLA-A*02: 06	53.84	0.7
			HLA-A*29: 02	34.54	0.2
			HLA-B*15: 02	85.48	0.1
			HLA-B*35: 01	11.17	0.2
			HLA-B*58: 01	193.7	0.5
			HLA-C*03: 03	46.15	0.4
			HLA-C*14: 02	25.99	0.2
LTSELTGTY	175	183	HLA-A*01: 01	9.72	0.1
			HLA-A*26: 01	97.31	0.1
			HLA-A*29: 02	76.29	0.2
			HLA-A*30: 02	61.26	0.2
			HLA-B*15: 01	64.37	0.2
			HLA-B*35: 01	26.26	0.3
LYYAFASIF	476	484	HLA-A*23: 01	18.34	0.2
			HLA-A*24: 02	29.1	0.4
			HLA-C*14: 02	9.85	0.1
MITETLSTF	367	375	HLA-A*02: 06	52.73	0.7
			HLA-B*15: 01	71.09	0.2
			HLA-B*35: 01	19.96	0.2
			HLA-B*53: 01	291.4	0.7

Table 7. The best epitopes of glycoprotein B that had binding affinity with the human MHC class I alleles

Peptide	Start	End	Allele	ic50	Percentile
FLFGSGYAL	598	606	HLA-A*02: 01	4.84	0.1
			HLA-A*02: 06	9.63	0.1
			HLA-B*15: 02	25.07	0.1
			HLA-B*39: 01	6.19	0.1
			HLA-C*03: 03	3.34	0.1
			HLA-C*12: 03	21.03	0.2
			HLA-C*14: 02	26.2	0.2
FMSNPFGAL	727	735	HLA-A*02: 01	89.13	0.2
			HLA-A*02: 06	10.59	0.2
			HLA-B*15: 01	64.38	0.1
			HLA-B*15: 02	84.88	0.1
			HLA-C*03: 03	4.33	0.1
			HLA-C*12: 03	47.63	0.2
			HLA-C*14: 02	49.07	0.2

3.3.2. MHC Class II Binding Predictions

T- Cell epitopes and interaction with MHC Class II were predicted based on the NN-align method with half maximal inhibitory concentration (IC50) ≤ 1000 nm. The most promising four epitopes of glycoprotein H and two of glycoprotein B that had binding affinity with human MHC class II alleles were shown in Table 8 and Table 9 repectively. The 3D structure of these epitopes was depicted in Figure 5.

Table 8. The best four epitopes of glycoprotein H that had binding affinity with the human MHC class II alleles

core sequence	peptide sequence	Start	End	Allele	IC50	Percentile
YVLRSAYAF	SILQHLYVLRSAYAF	419	433	HLA-DRB3*01: 01	67.9	3.47
	ILQHLYVLRSAYAFN	420	434	HLA-DPA1*01: 03/DPB1*02: 01	237.3	14.24
				HLA-DRB3*01: 01	73.1	3.66
	LQHLYVLRSAYAFNI	421	435	HLA-DRB3*01: 01	72.3	3.63
	QHLYVLRSAYAFNIS	422	436	HLA-DPA1*01: 03/DPB1*02: 01	218.3	13.56
				HLA-DPA1*02: 01/DPB1*01: 01	292	22.68
				HLA-DRB3*01: 01	85.9	4.06
	HLYVLRSAYAFNISQ	423	437	HLA-DPA1*01: 03/DPB1*02: 01	270.3	15.34
				HLA-DPA1*02: 01/DPB1*01: 01	278.5	22.03
				HLA-DRB3*01: 01	183.5	6.52
	LYVLRSAYAFNISQE	424	438	HLA-DPA1*01: 03/DPB1*02: 01	370.1	18.29
				HLA-DRB3*01: 01	345.6	9.46
	YVLRSAYAFNISQES	425	439	HLA-DPA1*01/DPB1*04: 01	864.4	20.17
				HLA-DRB3*01: 01	307.8	8.84
LTSELTGTY	EHRFILTSELTGTYV	170	184	HLA-DRB1*03: 01	447	13.18
	HRFILTSELTGTYVK	171	185	HLA-DQA1*05: 01/DQB1*03: 01	950.4	47.03
				HLA-DRB1*03: 01	311.6	10.58
				HLA-DRB5*01: 01	238.4	23.95
	RFILTSELTGTYVKH	172	186	HLA-DQA1*05: 01/DQB1*03: 01	944.4	46.9
				HLA-DRB1*03: 01	188.4	7.73
				HLA-DRB5*01: 01	219.1	22.98
	FILTSELTGTYVKHV	173	187	HLA-DRB1*03: 01	476.5	13.71
				HLA-DRB5*01: 01	438.8	31.3
	ILTSELTGTYVKHVC	174	188	HLA-DRB1*04: 01	870.7	39.88
				HLA-DRB5*01: 01	668.8	37.14
LYYAFASIF	TSARHALYYAFASIF	470	484	HLA-DPA1*01/DPB1*04: 01	316.2	11.35
				HLA-DPA1*02: 01/DPB1*01: 01	147.1	14.19
				HLA-DQA1*01: 01/DQB1*05: 01	424.3	8
	TSARHALYYAFASIF	470	484	HLA-DQA1*05: 01/DQB1*03: 01	103.3	15.15
				HLA-DRB1*01: 01	16.9	9.68
				HLA-DRB1*04: 01	174.3	13.39
				HLA-DRB1*04: 05	70.6	6.96
				HLA-DRB1*07: 01	9.6	1.54
				HLA-DRB1*09: 01	124.9	8.54
				HLA-DRB1*13: 02	854.9	23.74
				HLA-DRB3*01: 01	440.5	10.91
				HLA-DRB5*01: 01	46.2	9.46
	SARHALYYAFASIFQ	471	485	HLA-DPA1*03: 01/DPB1*04: 02	292.9	18.15
				HLA-DQA1*01: 01/DQB1*05: 01	316.4	6.37
				HLA-DQA1*05: 01/DQB1*03: 01	80	12.77

core sequence	peptide sequence	Start	End	Allele	IC50	Percentile
				HLA-DRB1*01: 01	13.3	7.53
				HLA-DRB1*04: 05	67.7	6.67
				HLA-DRB1*07: 01	9.9	1.62
				HLA-DRB1*09: 01	118.6	8.15
				HLA-DRB1*13: 02	693.3	21.06
				HLA-DRB3*01: 01	525.4	12.1
				HLA-DRB5*01: 01	32.8	7.36
	ARHALYYAFASIFQR	472	486	HLA-DQA1*01: 01/DQB1*05: 01	308.5	6.23
				HLA-DRB1*01: 01	9.9	5.11
				HLA-DRB1*04: 05	72.4	7.13
				HLA-DRB1*07: 01	13.3	2.41
				HLA-DRB1*08: 02	778.6	18.23
				HLA-DRB1*09: 01	112	7.7
				HLA-DRB1*13: 02	571.5	18.84
				HLA-DRB3*01: 01	649.8	13.73
	RHALYYAFASIFQRP	473	487	HLA-DQA1*01: 01/DQB1*05: 01	265.1	5.51
				HLA-DRB1*01: 01	11.6	6.39
				HLA-DRB1*04: 01	94.3	7.65
				HLA-DRB1*04: 05	94.5	9.16
				HLA-DRB1*07: 01	20.1	3.86
				HLA-DRB1*08: 02	676.4	16.12
				HLA-DRB1*09: 01	128.5	8.76
				HLA-DRB1*13: 02	657.5	20.43
	HALYYAFASIFQRPP	474	488	HLA-DQA1*01: 01/DQB1*05: 01	303.8	6.16
				HLA-DRB1*01: 01	19.9	11.25
				HLA-DRB1*07: 01	24.4	4.71
				HLA-DRB1*08: 02	712.9	16.91
				HLA-DRB1*09: 01	142.7	9.65
				HLA-DRB1*13: 02	923.1	24.79
	ALYYAFASIFQRPPN	475	489	HLA-DQA1*01: 01/DQB1*05: 01	405	7.72
				HLA-DRB1*01: 01	31.8	16.09
				HLA-DRB1*07: 01	38.6	6.99
	LYYAFASIFQRPPNE	476	490	HLA-DPA1*02: 01/DPB1*01: 01	23.3	2
				HLA-DQA1*01: 01/DQB1*05: 01	776.7	12.45
				HLA-DRB1*01: 01	51.4	21.58
				HLA-DRB1*07: 01	52	9
MITETLSTF	TEANIKMITETLSTF	361	375	HLA-DPA1*01: 03/DPB1*02: 01	453.6	20.41
				HLA-DPA1*02: 01/DPB1*01: 01	229.2	19.43
	TEANIKMITETLSTF	361	375	HLA-DPA1*03: 01/DPB1*04: 02	141.9	12.09
				HLA-DRB3*01: 01	358.7	9.67
	EANIKMITETLSTFA	362	376	HLA-DPA1*01/DPB1*04: 01	974.6	21.51
				HLA-DPA1*01: 03/DPB1*02: 01	385.4	18.7
				HLA-DPA1*02: 01/DPB1*01: 01	192.4	17.26
				HLA-DPA1*03: 01/DPB1*04: 02	88	8.88
				HLA-DRB3*01: 01	314.5	8.94
	ANIKMITETLSTFAL	363	377	HLA-DPA1*01: 03/DPB1*02: 01	309.6	16.56
				HLA-DPA1*02: 01/DPB1*01: 01	157.5	14.94
				HLA-DPA1*03: 01/DPB1*04: 02	53.6	6.11
				HLA-DRB3*01: 01	246.7	7.76
	NIKMITETLSTFALH	364	378	HLA-DPA1*01: 03/DPB1*02: 01	286	15.84
				HLA-DPA1*02: 01/DPB1*01: 01	129.6	12.87
				HLA-DPA1*03: 01/DPB1*04: 02	52.3	5.99
				HLA-DRB3*01: 01	261.7	8.04
	IKMITETLSTFALHS	365	379	HLA-DPA1*01: 03/DPB1*02: 01	318.9	16.85
				HLA-DPA1*02: 01/DPB1*01: 01	131.6	13.02
				HLA-DPA1*03: 01/DPB1*04: 02	64.2	7.02
				HLA-DRB3*01: 01	672.3	14
	KMITETLSTFALHSN	366	380	HLA-DPA1*01: 03/DPB1*02: 01	470	20.79
				HLA-DPA1*02: 01/DPB1*01: 01	182.3	16.63
				HLA-DPA1*03: 01/DPB1*04: 02	105.7	10.04
	MITETLSTFALHSNP	367	381	HLA-DPA1*01: 03/DPB1*02: 01	708.5	25.7
				HLA-DPA1*03: 01/DPB1*04: 02	220.1	15.6
				HLA-DRB1*04: 04	265.8	24.43

Table 9. The best two epitopes of glycoprotein B that had binding affinity with the human MHC class II alleles

Core Sequence	Peptide Sequence	Start	End	Allele	IC50	Rank
FMSNPFGAL	ISGVSAFMSNPFGAL	721	735	HLA-DPA1*01/DPB1*04: 01	18.5	1.18
	ISGVSAFMSNPFGAL			HLA-DPA1*01: 03/DPB1*02: 01	11.6	1.36
	ISGVSAFMSNPFGAL			HLA-DPA1*02: 01/DPB1*01: 01	107.9	11.08
	ISGVSAFMSNPFGAL			HLA-DPA1*03: 01/DPB1*04: 02	307.3	18.61
	ISGVSAFMSNPFGAL			HLA-DRB3*01: 01	217.7	7.21
	ISGVSAFMSNPFGAL			HLA-DRB5*01: 01	332.7	27.83
	SGVSAFMSNPFGALA	722	736	HLA-DPA1*01/DPB1*04: 01	11.8	0.69
	SGVSAFMSNPFGALA			HLA-DPA1*01: 03/DPB1*02: 01	10.5	1.21
	SGVSAFMSNPFGALA			HLA-DPA1*02: 01/DPB1*01: 01	96.7	10.1
	SGVSAFMSNPFGALA			HLA-DPA1*03: 01/DPB1*04: 02	182.4	14.04
	SGVSAFMSNPFGALA			HLA-DQA1*05: 01/DQB1*03: 01	217	23.62
	SGVSAFMSNPFGALA			HLA-DRB3*01: 01	191.6	6.69
	SGVSAFMSNPFGALA			HLA-DRB5*01: 01	351.5	28.49
	GVSAFMSNPFGALAI	723	737	HLA-DPA1*01/DPB1*04: 01	10.1	0.56
	GVSAFMSNPFGALAI			HLA-DPA1*01: 03/DPB1*02: 01	9.4	1.05
	GVSAFMSNPFGALAI			HLA-DPA1*02: 01/DPB1*01: 01	83	8.82
	GVSAFMSNPFGALAI			HLA-DPA1*03: 01/DPB1*04: 02	91.3	9.09
	GVSAFMSNPFGALAI			HLA-DRB3*01: 01	147	5.69
	GVSAFMSNPFGALAI			HLA-DRB5*01: 01	266	25.2
	VSAFMSNPFGALAIG	724	738	HLA-DPA1*01/DPB1*04: 01	10.6	0.6
	VSAFMSNPFGALAIG			HLA-DPA1*01: 03/DPB1*02: 01	10.3	1.18
	VSAFMSNPFGALAIG			HLA-DPA1*02: 01/DPB1*01: 01	95.3	9.98
	VSAFMSNPFGALAIG			HLA-DPA1*03: 01/DPB1*04: 02	85.8	8.72
	VSAFMSNPFGALAIG			HLA-DRB1*01: 01	46	20.25
	VSAFMSNPFGALAIG			HLA-DRB3*01: 01	171.5	6.26
	VSAFMSNPFGALAIG			HLA-DRB5*01: 01	302.6	26.7
	SAFMSNPFGALAIGL	725	739	HLA-DPA1*01/DPB1*04: 01	15.8	0.99
	SAFMSNPFGALAIGL			HLA-DPA1*01: 03/DPB1*02: 01	17.5	2.1
	SAFMSNPFGALAIGL			HLA-DPA1*02: 01/DPB1*01: 01	209.4	18.3
	SAFMSNPFGALAIGL			HLA-DPA1*03: 01/DPB1*04: 02	120.7	10.93
	SAFMSNPFGALAIGL			HLA-DRB1*01: 01	66.9	24.84
	SAFMSNPFGALAIGL			HLA-DRB3*01: 01	420.4	10.6
	SAFMSNPFGALAIGL			HLA-DRB5*01: 01	453.2	31.74
	AFMSNPFGALAIGLI	726	740	HLA-DPA1*01/DPB1*04: 01	63.8	3.75
	AFMSNPFGALAIGLI			HLA-DPA1*01: 03/DPB1*02: 01	67.2	6.36
	AFMSNPFGALAIGLI			HLA-DPA1*02: 01/DPB1*01: 01	268.2	21.51
	AFMSNPFGALAIGLI			HLA-DPA1*03: 01/DPB1*04: 02	144	12.19
	AFMSNPFGALAIGLI			HLA-DRB5*01: 01	560.2	34.62
	FMSNPFGALAIGLII	727	741	HLA-DPA1*01/DPB1*04: 01	243.5	9.65
	FMSNPFGALAIGLII			HLA-DPA1*01: 03/DPB1*02: 01	215.7	13.47
	FMSNPFGALAIGLII			HLA-DPA1*02: 01/DPB1*01: 01	309.8	23.51
	FMSNPFGALAIGLII			HLA-DPA1*03: 01/DPB1*04: 02	272.7	17.49
FLFGSGYAL	ANHRRYFLFGSGYAL	592	606	HLA-DPA1*01/DPB1*04: 01	132.6	6.46
	ANHRRYFLFGSGYAL			HLA-DPA1*01: 03/DPB1*02: 01	78.1	7.07
	ANHRRYFLFGSGYAL			HLA-DPA1*02: 01/DPB1*01: 01	180.5	16.51
	ANHRRYFLFGSGYAL			HLA-DQA1*05: 01/DQB1*03: 01	27.2	5.18
	ANHRRYFLFGSGYAL			HLA-DRB1*01: 01	4.8	0.62
	ANHRRYFLFGSGYAL			HLA-DRB1*04: 01	157.7	12.31
	ANHRRYFLFGSGYAL			HLA-DRB1*07: 01	39.9	7.18
	ANHRRYFLFGSGYAL			HLA-DRB1*09: 01	355.4	20.17
	ANHRRYFLFGSGYAL			HLA-DRB1*13: 02	822.8	23.24
	NHRRYFLFGSGYALF	593	607	HLA-DQA1*05: 01/DQB1*03: 01	18	3.27
	NHRRYFLFGSGYALF			HLA-DRB1*01: 01	4.4	0.32
	NHRRYFLFGSGYALF			HLA-DRB1*04: 01	129.3	10.34
	NHRRYFLFGSGYALF			HLA-DRB1*07: 01	39.8	7.17

NHRRYFLFGSGYALF			HLA-DRB1*09: 01	204.3	13.18
NHRRYFLFGSGYALF			HLA-DRB1*13: 02	589.4	19.17
HRRYFLFGSGYALFE	594	608	HLA-DQA1*05: 01/DQB1*03: 01	13.7	2.3
HRRYFLFGSGYALFE			HLA-DRB1*01: 01	4.2	0.19
HRRYFLFGSGYALFE			HLA-DRB1*04: 01	124.9	10.01
HRRYFLFGSGYALFE			HLA-DRB1*07: 01	48.3	8.47
HRRYFLFGSGYALFE			HLA-DRB1*09: 01	122.8	8.41
HRRYFLFGSGYALFE			HLA-DRB1*13: 02	495.6	17.32
HRRYFLFGSGYALFE			HLA-DRB5*01: 01	67.9	12.18
RRYFLFGSGYALFEN	595	609	HLA-DQA1*05: 01/DQB1*03: 01	13	2.14
RRYFLFGSGYALFEN			HLA-DRB1*01: 01	4.1	0.13
RRYFLFGSGYALFEN			HLA-DRB1*04: 01	117.4	9.45
RRYFLFGSGYALFEN			HLA-DRB1*07: 01	72.5	11.42
RRYFLFGSGYALFEN			HLA-DRB1*13: 02	419.6	15.66
RRYFLFGSGYALFEN			HLA-DRB5*01: 01	87.3	14.15
RYFLFGSGYALFENY	596	610	HLA-DQA1*05: 01/DQB1*03: 01	13.3	2.21
RYFLFGSGYALFENY			HLA-DRB1*01: 01	4.8	0.62
RYFLFGSGYALFENY			HLA-DRB1*04: 01	157.5	12.3
RYFLFGSGYALFENY			HLA-DRB1*07: 01	108.3	14.94
RYFLFGSGYALFENY			HLA-DRB1*13: 02	579.2	18.98
RYFLFGSGYALFENY			HLA-DRB5*01: 01	164.5	19.95
YFLFGSGYALFENYN	597	611	HLA-DQA1*05: 01/DQB1*03: 01	14.1	2.39
YFLFGSGYALFENYN			HLA-DRB1*01: 01	6.3	1.99
YFLFGSGYALFENYN			HLA-DRB1*04: 01	169.4	13.08
YFLFGSGYALFENYN			HLA-DRB1*07: 01	169.5	19.55
YFLFGSGYALFENYN			HLA-DRB1*13: 02	912.5	24.63
FLFGSGYALFENYNF	598	612	HLA-DQA1*05: 01/DQB1*03: 01	19.9	3.68
FLFGSGYALFENYNF			HLA-DRB1*01: 01	9	4.39
FLFGSGYALFENYNF			HLA-DRB1*07: 01	164.8	19.27

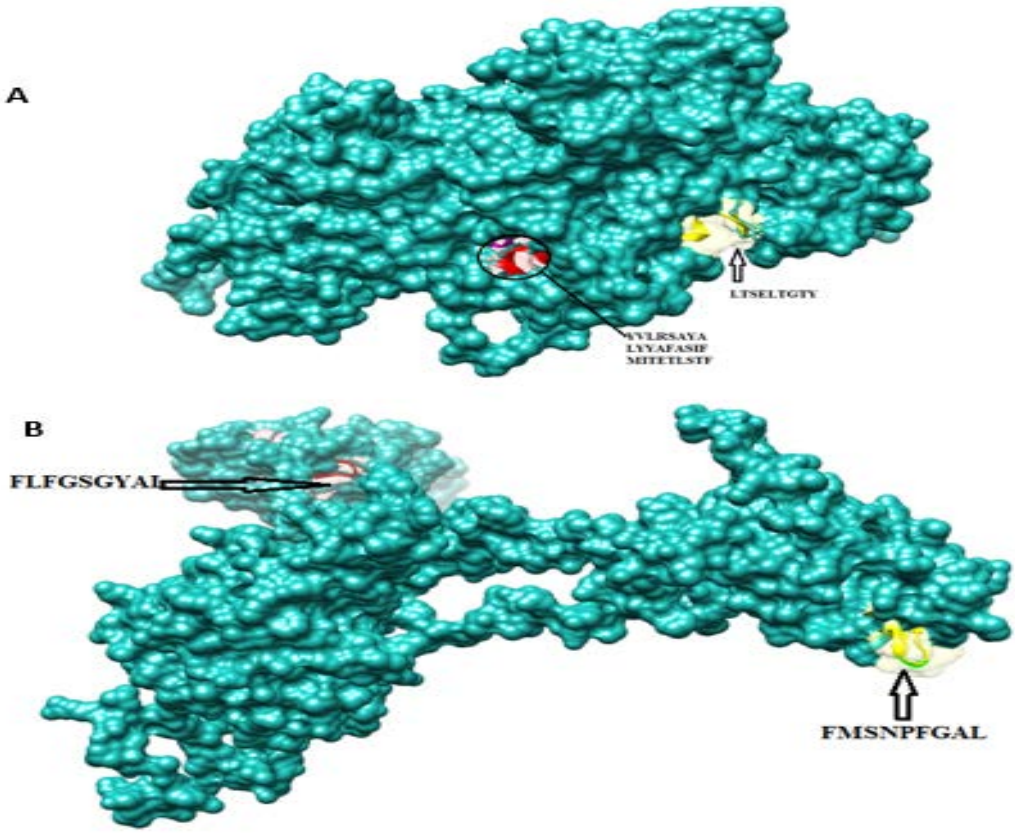

Figure 5. Structural position of the most promising conserved T- cell epitopes. A: glycoprotein H and B: glycoprotein B of MDV-1 that Interacts with both MHC-I and MHC-II alleles

3.3.3. Molecular Docking of the Proposed Epitopes with MHC Class 1 Alleles

Four docked epitopes; $_{425}$-**YVLRSAYAF**-$_{433}$, $_{175}$-**LTSELTGTY**-$_{183}$, $_{476}$-**LYYAFASIF**-$_{484}$ and $_{367}$-**MITETLSTF**-$_{375}$ of glycoprotein H and two epitopes; $_{598}$-**FLFGSGYAL**-$_{606}$ and $_{727}$-**FMSNPFGAL**-$_{735}$ of glycoprotein B were found to have binding affinity to chickens MHC I molecule (BF2*2101) haplotype which the binding energy score for four epitopes as shown in Table 10 as obtained from patch dock. Figure 6 visualize the binding interactions between MHC I receptor and epitopes in the structural level.

Table 10. Docking results of most promising predicted and modeled MHC-I binding epitopes with the binding energy score.

Epitope	Start	End	Binding Energy score (kcal/mol)
Epitope of glycoprotein H			
YVLRSAYAF	425	433	-60.58
LTSELTGTY	175	183	-68.56
LYYAFASIF	476	484	-72.45
MITETLSTF	367	375	-49.52
Epitope of glycoprotein B			
FLFGSGYAL	598	606	-54.25
FMSNPFGAL	727	735	-58.44

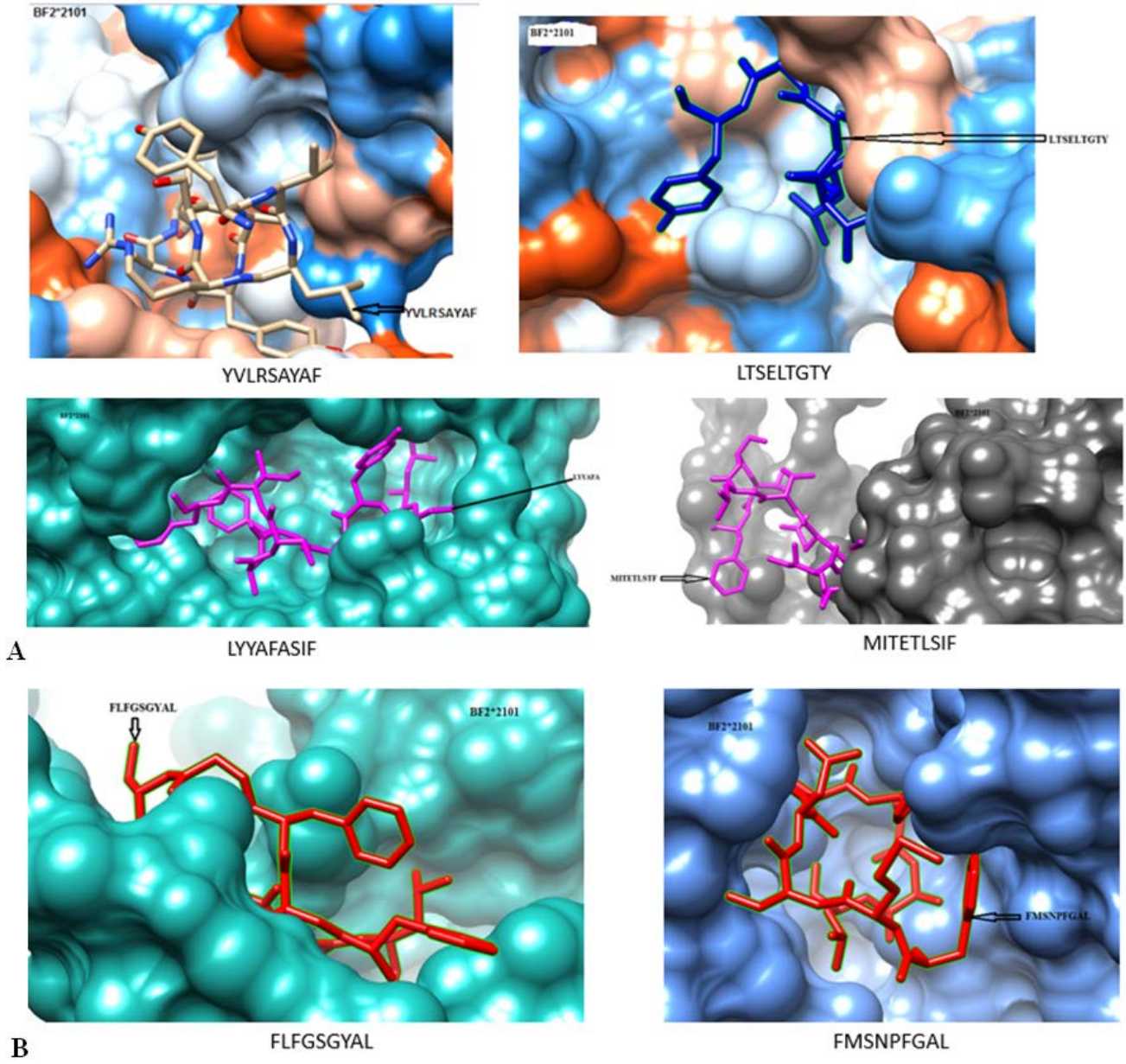

Figure 6. Interaction of proposed epitopes with MHC I allele of Chickens (BF2*2101). **A**: Epitopes of glycoprotein H. **B**: epitopes of glycoprotein B

4. Discussion

Vaccination is usually considered to be the most effective method of preventing infectious diseases. Inactivated vaccines live attenuated vaccines, Subunit vaccines, and DNA vaccines were shown with drawbacks. They were characterized by time-consuming process and active infectious particles can be administered together with the vaccine [46]. The increase of incidence of viral infections in animals and human provided the need of new available technologies. For instance the peptides based vaccines approach enables achieving effective, cost-efficient vaccines development. The process is based on identification and chemical synthesis of B-cell and T-cell epitopes that can induce specific immune responses [45].

Therefore, in this study, we aimed to design epitopes based vaccine for Marek's disease virus serotype 1 (MDV-1). Marek's disease (MD) caused by Marek's disease virus (MDV) serotype 1 (MDV-1) is oncogenic which causes economic losses in poultry industry estimated to be more than 1$ billion per year [10,26,68]. In this report both glycoprotein H and B of Marek's disease were targeted as an immunogens and tested for their efficacy in eliciting immunity against B-cell and T-cell. In our results three epitopes of glycoprotein H ($_{91}$-**FYKRPVSKLL**-$_{100}$, $_{255}$-**LKPYEPVDKF**-$_{264}$, and $_{684}$-**PRPL**-$_{687}$) and three epitopes of glycoprotein B ($_{162}$-**EKQV**-$_{165}$, $_{234}$-**YGLSPPE**-$_{240}$, and $_{363}$-**YNDSHVK**-$_{369}$) were shown to elicit the B cells. They fulfilled the criteria of surface accessibility, antigenicity. Therefore they were proposed as B cell epitopes since they got scores above thresholds in Bepipred, Emini surface accessibility and Kolaskar and Tongaonkar antigenicity prediction methods and showed 100% conservancy.

During virus infection, the importance of MHC I and II in fighting the infection is crucial [69]. Most importantly since there were no data available in the IEDB concerning chicken alleles, the human alleles were used to predict the allelic interaction with MHC1 and MHC11. Several studies concluded the highly similarities between human and chickens MHC molecules [60,62,63,70]. Interestingly four epitopes namely ($_{425}$-**YVLRSAYAF**-$_{433}$, $_{175}$-**LTSELTGTY**-$_{183}$, $_{476}$-**LYYAFASIF**-$_{484}$ and $_{367}$-**MITETLSTF**-$_{375}$) from glycoprotein H and two epitopes namely ($_{598}$-**FLFGSGYAL**-$_{606}$ and $_{727}$-**FMSNPFGAL**-$_{735}$) from glycoprotein B were found to interact with high binding affinity to both MHC1 and MHC11 alleles. Therefore these epitopes were selected as promising peptides vaccine against Marek's disease. Furthermore for critical binding the predicted epitopes were further docked against MHC1 molecule. Strikingly the predicted epitopes from the glycoprotein H and B demonstrated low binding energy score to chickens MHC class I molecule (BF2*2101) haplotype in the structural level. This result could further solidify the ability of the predicted epitopes to act as strong vaccine epitopes

To our knowledge there is no epitope based vaccine for the Marek's disease virus serotype 1 (MDV-1) using in silico approach. The advantages of this approach is focusing in immune response, enhancing immunity and reducing costs [47,71]. It is noteworthy the peptide based vaccine approach have successfully used in therapeutic and designing peptide-based vaccine in a considerable number of human and animal viruses and diseases such as Influenza virus, Paratuberculosis, HIV, Ebola virus, cancer and others [72-78].

This study proposed an interesting epitopes of glycoprotein H and glycoprotein B that have very strong binding affinity to both B and T cells (MHC1 and MHC11 alleles) which indicates strong potential to formulate peptide vaccine for Marek's disease virus serotype (MDV-1). An in vitro and in vivo application is required to prove the efficacy of the predicted epitopes as peptide vaccine.

5. Conclusion

In conclusion, this study indicated that immunoinformatics wide screening of vaccine targets of emerging highly pathogenic pathogens is a promising strategy to accelerate their vaccine development, which lessens the time and cost required for laboratory analysis of pathogen gene products.

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Competing Interest

The authors declare that they have no competing interests.

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