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In Silico Design of Epitope Based Peptide Vaccine against Virulent Strains of (HN)-Newcastle Disease Virus (NDV) in Poultry Species

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Abstract

*Newcastle disease virus (NDV) belongs to the family Paramyxoviridae. It causes Newcastle disease, mainly by the virulent strains of avian paramyxovirus serotype-1 (AMPV-1). This virus represents a huge problem on the world's economy than any other animal virus, especially in developed countries. ND infection is not obstructed and many studies have attributed the spread of the disease to changes in the genome of the virus and emersion to new strains. We aimed to design a peptide vaccine for NDV particularly for the haemagglutininneuraminidase protein (HN) using computational methods to predict epitopes inducing immune system and can be used later to create a new peptide vaccine could replace conventional vaccines. A total of available 60 virulent strains of HN- NDV were retrieved from NCBI for bioinformatics analysis using Immune Epitope Data Base (IEDB) to predict B and T cells Epitopes. We used human MHC class I and II alleles in this study due to the difficulty to determine MHC B complex alleles in Poultry. Then we docked the best predicted CTL epitopes with B-F alleles (BF2*2101 and BF2*0401). Four CTL cell epitopes namely (^{548}**ISNTLFGEF**^{556}, ^{546}**AEISNTLFG**^{554}, ^{88}**VALESPLAL**^{96} and ^{526}**YTTSTCFKV**^{534}) interacted with MHC class (B-F) I alleles and we suggested them to become universal peptides based vaccine against NDV. We found these CTL epitopes to be T helper epitopes also. The overlapping between MHC class I (B-F) and (B-L) II T cell epitopes suggesting the possibility of antigen presentation to immune cells via both MHC class I and II pathways especially the overlapping between ^{548}**ISNTLFGEF**^{556} and ^{546}**AEISNTLFG**^{554}. We considered this study distinctive because no research ever dealt with peptide based vaccine on virulent strains of NDV using in silico approach.*

Keyword: *Newcastle disease virus (NDV), poultry, MHC B complex and vaccine.*

1. Introduction

Newcastle disease virus (NDV) is one of the species that belongs to the genus Avulavirus, order Mononegavirales, family Paramyxoviridae. It is an enveloped virus with a negative sense, non-segmented, single stranded RNA genome [1-3]. Viral genome consists of six viral structural proteins.

Respectively they are: a nucleoprotein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), haemagglutininneuraminidase (HN), and large protein (L). However, the F gene and the HN gene are the essential proteins in virulence determination. The fusion (F) protein is responsible for mediating fusion of the viral envelope with cellular membranes while the haemagglutinin-neuraminidase (HN) protein is involved in cell attachment and release [4-7].

NDV which has ability to infect more than 240 species of birds worldwide, caused Newcastle disease mainly by the virulent strains of avian paramyxovirus serotype-1 (AMPV-1). However, NDV can be harmful to human beings as it can cause eye conjunctivitis but it is rare [1, 8]. NDV was classified according to pathotyping assays to three classes' virulent (velogenic), moderately virulent (mesogenic), and non-virulent (lentogenic) [9, 10]. The major singes of Newcastle disease include respiratory distress, diarrhea, circulatory failure, and central nervous system impairment, but infection in human is rare and appearance as eye conjunctivitis [6, 8, 11].

The issue of Newcastle disease (ND) has received considerable critical attention since the 1926s, on which the first outbreak occurred in Java, Indonesia, and Newcastle-upon-Tyne, England [7]. Previous studies have reported, there has been a dramatic outbreak of ND globally [1, 9, 12, 13], precisely in Asia, Central and South America and in most African Countries [8, 14]. This virus represents a huge problem on the world's economy than any other animal virus, especially in developed countries like Sudan [3,9,15-16].

Immunity in Vertebrates "including chickens" basically has two types of defense systems against infectious agents; Innate (non-specific) and adaptive (specific)

immunity. Specific immunity encompasses two aspects of immunity. These are humoral immunity, which is carried out by antibodies, and cell-mediated immunity which is carried out by T cells [17,18]. Chicken lymphocytes consist of B cells which develop in the bursa of Fabricius, and T cells that develop in the thymus. Both B and T cells express antigen receptors on their surface [18]. Regulation of cellular communication in the immune response is a critical function of the chicken MHC (major histocompatibility) [19]. The chicken MHC (B-complex) gene cluster, located on a microchromosome (chromosome 16) contains class I (B–F) and class Ⅱ (B-LB) genes that are similar to those of the mammalian species in the encoded protein structure [20]. This MHC was likely responsible for the appearance of the antigen presentation pathways and receptor–ligand interactions of the adaptive immune system and there are strong associations of the chicken MHC with disease resistance and vaccine response [19-21]. B lymphopoiesis is a regulated process that occurs in the bone marrow of humans and rodents and gut-associated lymphoid tissue (GALT) of other mammals and birds to generate protection against pathogens. And the activation of chicken B cell to antibodies secretion required interaction between CD40 on the B cell surface and CD40 ligand in the helper T cell. This process appears to be indistinguishable from the activation of mammalian B cell [22-24].

Despite the presence of Vaccines for chickens, like inactivated vaccines and attenuated live vaccines which preserve them from morbidity and mortality, ND infection is not obstructed and many studies have attributed the spread of the disease to changes in the genome of the virus and emersion to new strains [5, 12, 25-27]. Vaccines are mostly based on B cell immunity, but recently, vaccines based on T cell epitope have been encouraged as the host can generate a strong immune response by CD8+ T cell against the infected cell [28]. Moreover, *in silico* approach has become handy in vaccine designing as it provides clue to select target protein sequence. In addition, vaccine failure from many countries on the ability of classical NDV vaccines to stop spread of disease was reported [29,30].

In this paper we aimed to design a peptide vaccine for NDV particularly for the haemagglutininneuraminidase protein (HN) using computational methods to predict epitopes inducing positive, desirable T cell and B cell mediated immune response and can be used later to create a new peptide vaccine that could replace conventional vaccines depending on *in silico* approaches and information in database. We considered this study distinctive because no research dealt with peptide based vaccine against virulent strains of NDV by *in silico* approach.

Table 1 Virulent strains of HN-NDV and their Accession numbers, host date and area of collection

Accession No.	Strain Name	Country	Host	Date of Collection
*NP_071470.1	B1	USA	Unknown	N/A
P12553.1	Unknown	N/A	Unknown	N/A
AHJ81382.1	NDV/crested ibis/China/Shaanxi10/2010	China	Ibis	2010
AHJ81376.1	NDV/crested ibis/China/Shaanxi06/2006	China	Ibis	2006
ACW19918.1	JSD0812	China	Duck	2008
ACU30817.1	NDV/Chicken/Egypt/1/2005	Egypt	Chicken	2005
ABQ14808.1	JG97	China	Goose	N/A
AJE26307.1	Chicken/Pak/Quality Operations Lab/Sfr-611/13	Pakistan	Chicken	2013
AHJ57315.1	Apmv-1/Chicken/Brazil/Sjm/75	Brazil	Chicken	N/A
AHZ89383.1	SF2	China	Unknown	2010
AHV78501.1	Belize (Spanish Lookout)/4224-3/2008	Belize	Chicken	2008
AFY07433.1	01-Oct	Mexico	Chicken	2010
AFK25763.1	9a5b-D5c1	China	Unknown	2009
AEX01230.1	Poultry/Peru/1918-03/2008	Peru	Poultry	2008
AEA10388.1	GD09-2	China	Duck	2009
AEM55587.1	JSG0210	China	Goose	2002
ADZ45537.1	Chicken_Sweden_95	Sweden	Chicken	1995
ADG27333.1	APMV-1/Chicken/U.S.(Tx)/Gb/1948	USA	Chicken	1948
ADD82463.1	Ndv/Wb/Ch/Hlj001/06	China	Bird	2006
ADD82462.1	Ndv/Md/Ch/Hlj016/06	China	Duck	2006
ADD82461.1	NDV/mallard/CH/HLJ383/06	China	Duck	2006
ADD82460.1	NDV/mallard/CH/HLJ374/06	China	Duck	2006
ADD82459.1	NDV/mallard/CH/HLJ363/05	China	Duck	2005
ADD82458.1	NDV/mallard/CH/HLJ361/05	China	Duck	2005
ADD82457.1	NDV/mallard/CH/HLJ355/06	China	Duck	2006
ADD82456.1	NDV/mallard/CH/HLJ128/06	China	Duck	2006
ADD82455.1	NDV/mallard/CH/HLJ127/06	China	Duck	2006
ADD82454.1	NDV/AG/CH/HLJ070/06	China	Sparrow hawk	2006
ADD82453.1	NDV/WfG/CH/HLJ052/06	China	Goose	2006
ADD82452.1	NDV/MD/CH/HLJ028/06	China	Duck	2006

ADD82451.1	NDV/buzzard/CH/HLJ013/06	China	Hawk	2006
ADD82450.1	NDV/owl/CH/HLJ012/06	China	Owl	2006
ADD82449.1	NDV/buzzard/CH/HLJ009/06	China	Hawk	2006
ACI62502.1	GO/CH/HLJ/LL01/08	China	Goose	2008
ABS84265.1	Gamefowl/Us(Ca)/212676/2002	USA	Fowl	2002
ABS84263.1	Pigeon/Ny/Us/1984	USA	Pigeon	1984
ABS84262.1	Northern Pintail/Us(Ak)/196/1998	USA	Duck	1998
AEA10394.1	Sd09	China	Duck	2009
AFV46455.1	Wf00g	China	Goose	2000
AFV46450.1	Wf00d	China	Duck	2000
AEZ00712.1	APMV-1/Chicken/Nl/152608/93	Netherlands	Chicken	1993
ABM89487.1	871	Germany	Parrot	N/A
ABM89484.1	472	Germany	Parrot	N/A
AEZ00724.1	PPMV-1/Pigeon/Ie/806/04	Ireland	Pigeon	2004
AEZ00718.1	APMV-1/Chicken/Za/Al495/04	South Africa	Goose	2004
AEZ00706.1	APMV-1/Chicken/Ca/2098/71	USA	Chicken	1971
ABM89499.1	626	Germany	Cockatoo	N/A
ABM89496.1	625	Germany	Macaw	N/A
ABM89493.1	624	Germany	Parrot	N/A
ABM89490.1	599	Germany	Parrot	N/A
AAQ54642.1	Turkey/Usa(Nd)/43084/92	N/A	Unknown	N/A
AAQ54636.1	Cockatoo/Indonesia/14698/90	Indonesia	Unknown	N/A
AAQ54634.1	Chicken/Kenya/139/90	Kenya	Unknown	N/A
AAQ54632.1	Pigeon/Italy/1166/00	N/A	Unknown	N/A
AAQ54630.1	Dove/Italy/2736/00	Italy	Unknown	N/A
AAQ54626.1	Chicken/Honduras/15/00	N/A	Unknown	N/A
AAQ54624.1	Chicken/Usa(Ca)/1083(Fontana)/72	N/A	Unknown	N/A
AKT71960.1	Chicken/Ndv/Pak/Aw-14	Pakistan	Chicken	2014
AAA46672.1	Unknown-M32415	N/A	Unknown	N/A
AAA46669.1	Unknown-M21409	N/A	Unknown	N/A

*Reference strain

2. Material and Methods

2.1 Protein Sequence Retrieval

A total of available 60 virulent strains of HN-Newcastle disease Virus (NDV) from different geographic regions were retrieved from the NCBI database (http://www.ncbi.nlm.nih.gov/protein/?term=hn+newcastle+disease+virus+virulent) in April 2016 and selected for immunobioinformtics analysis. These strains were isolated from different poultry species including: Ibis, Goose, Chicken, Duck, Bird, Sparrow hawk, Hawk, Owl, Fowl, Pigeon, Parrot, Cockatoo and Macaw. In this study haemagglutininneuraminidase (HN) was selected from six structural proteins for epitopes prediction based on NCBI Reference Sequence (NP_071470.1). Retrieved Strains and their Accession numbers and geographical regions are listed in Table (1).

2.2 Identification of Conserved Regions

Retrieved sequences were aligned using multiple sequence alignments (MSA) through BioEdit software [31]. 100% of identical and similar amino acid sequences were selected as a conserved region. That conserved region was then used for B and T cells' epitopes prediction using Immune Epitope Data Base (IEDB) servers (http://www.iedb.org/) based on the physico-chemical properties of a non-redundant dataset and artificial neural network [32,33].

2.3 Sequence-based method

2.3.1 T cell Epitope Prediction

The major histocompatibility complex MHC class-I binding prediction tool (http://tools.iedb.org/mhci/) [34] was used to predict Cytotoxic T cell epitopes using MHC class-I alleles (HLA A, B and C in man) based on Stabilized Matrix Method (SMM) [29] and percentile rank ≤1 .

The MHC class-II binding prediction tool (http://tools.iedb.org/mhcii/) [35] was used to predict helper T-cell epitopes. The percentile rank for strong binding peptides was set at ≤10 to determine the interaction potentials of helper T-cell epitope peptide and MHC class II allele (HLA DR, DP and DQ) [36].

MHC class I and II alleles were used in this study due to the difficulty to determine MHC B complex alleles in Poultry.

2.3.2. B cell Epitope Prediction

Continuous B cell epitope prediction tools of IEDB (http://toolsiedb.ofg/bcell/) were predicted depending on five algorithms: Bepipred predicts linear B-cell epitopes using hidden Markov model [37] with default threshold 0.35, Emini surface accessibility prediction tool [38] with default threshold 1.000, Kolaskar and Tongaonkar

Antigenicity prediction tool [39] was used also in this study with default threshold of 1.034, parker hydrophilicity prediction tool [40] with default threshold 1.549 and finally Chou & Fasman Beta-Turn Prediction tool [41] was used for more confirmation with default threshold 1.008.

2.4 Structure-based methods

2.4.1 Visualization of 3D Structures of Predicted Epitopes

Three dimensional (3D) structure and binding residues of HN protein was performed by **P**rotein **H**omology/analog**Y** **R**ecognition **E**ngine V 2.0 (phyre2) server (http://www.sbg.bio.ic.ac.uk/phyre2) [42] and UCSF-Chimera visualization tool 1.8 [43] to detect the location and to visualize the predicted B and T lymphocyte epitopes in the structural level.

2.4.2 Molecular Docking

Two chicken BF alleles /receptors (BF2 *2101, BF2*0401) have been evaluated according to peptide-binding groove affinity which reported by Kokh M. *et al.* (2007) & Zhang J. *et al.* (2012) [44,45]. The homology modeling in 3D structure of ligands (predicted epitopes) has been done with PEP STR MOD-Structure Prediction of Peptides Containing Natural, Non-Natural and Modified Residue (http://osddlinux.osdd.net/raghava/pepstrmod/nat_ss.php) [46]. While 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) [47]. A molecular docking technique was applied using Python Prescription (PyRx) -Virtual Screening Tool (http://sourceforge.net/projects/pyrx/) [48] to model the interactions of the 3D-modeled epitopes with BF alleles. Then the visualization has done by The PyMOL Molecular Graphics System, Version 1.8, Schrödinger LLC [49] (https://www.pymol.org/) and UCSF-Chimera visualization tool 1.8 [50]. Re-docking the results was done by MTiAutoDock 1.0 (http:// mobyle .rpbs.univ-parisdiderot.fr/cgibin/portal.py#forms::MTiAutoDock) [51] and PatchDock (http://bioinfo3d.cs.tau.ac.il/PatchDock/) online auto-dock tools for more confirmation [52].

3.1 Conservancy

The percentage of the peptides' conservancy in this study was 100%.

3.2 Prediction of Cytotoxic T-lymphocyte epitope and interaction with MHC Class I and Modeling

HN protein analyzed using IEDB MHC-1 binding prediction tool to predict cytotoxic T cell epitope interacted with different types of MHC Class I alleles in man. Based on Consensus SMM method with percentile rank ≤1; four conserved peptides were predicted to interact with selected human's MHC-1 alleles. The peptide ^{548}**ISNTLFGEF**^{556} interacted with two human alleles: HLA-B*44:02 and HLA-B*15:01. The other peptides ^{546}**AEISNTLFG**^{554}, ^{526}**YTTSTCFKV**^{534} and ^{88}**VALESPLAL**^{96} were interacted with one allele only and the results are listed in Table (2), Figure (1) at the structural level.

Table.2 list of CTL's epitopes that had binding affinity with the Human MHC Class I alleles instead of B-F alleles

peptide	start	end	length	allele	percentile rank	smm_ic50
AEISNTLFG	546	554	9	HLA-B*44:02	0.45	318.13
ISNTLFGEF	548	556	9	HLA-B*46:01	0.55	1298.02
				HLA-B*15:01	1	149.97
YTTSTCFKV	526	534	9	HLA-A*68:02	0.7	40.51
VALESPLAL	88	96	9	HLA-B*46:01	0.75	2340.35

Percentile rank ≤ 1

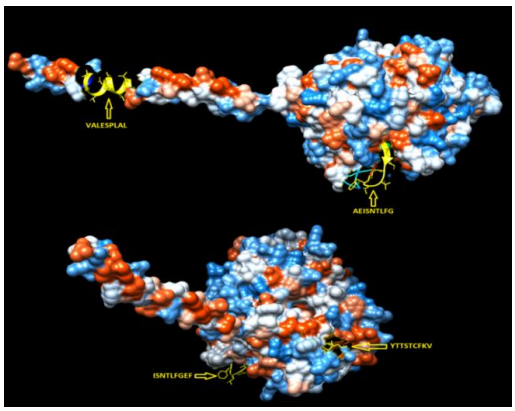

Figure.1 3D structure of proposed cytotoxic T cell epitopes suggested to be interact with MHC I of HN protein in virulent strains of NDV virus illustrated by UCSF-Chimera visualization tool

3.3 Prediction of T helper cell epitope and interaction with MHC Class II

A total of 12 conserved peptides with 9-mer core sequences in the HN protein of NDV were identified to be helper T-cell epitopes using the IEDB MHC-1 binding prediction tool with percentile rank ≤ 10 were found to be interacted with different forms of Human's alleles of (HLA-DRB1, HLA-DRB3, HLA-DRB5, HLA-DPA1/HLA-DPB1 and DQA1/ DQB1). Among these 12 epitopes; a 9-mer epitope: ^{548}**ISNTLFGEF**^{556} had affinity to interact with 11 alleles (HLA-DPA1*01:03/DPB1*02:01, HLA-DPA1*01/DPB1*04:01, HLA-DPA1*02:01/DPB1*01:01, HLA-DRB3*01:01, HLA-DPA1*02:01/DPB1*05:01 and HLA-DPA1*03:01/DPB1*04:02) followed by ^{484}**LRGVFGTML**^{492205} and **YLALGVLRT**^{213} epitopes had affinity to interact with 9 and 7 alleles **(**HLA-DRB1*07:01, HLA-DRB5*01:01, HLA-DRB1*11:01, HLA-DRB1*08:02,

HLA-DRB1*04:05, HLA-DRB1*09:01, HLA- DRB1*01:01, HLA-DRB1*15:01 and HLA-DRB1*03:01) and (HLA-DRB1*11:01, HLA-DRB1*04:01, HLA-DPA1*03:01/DPB1*04:02, HLA-DRB1*01:01, HLA-DRB1*04:05, HLA-DRB1*15:01 and HLA-DRB1*13:02), respectively. ^{548}**ISNTLFGEF**^{556} had affinity to interact with 6 alleles (HLA-DPA1*01:03/DPB1*02:01, HLA-DPA1*01/DPB1*04:01, HLA-DPA1*02:01/DPB1*01:01, HLA-DRB3*01:01, HLA-DPA1*02:01/DPB1*05:01 and HLA-DPA1*03:01/ DPB1*04:02), Table (3).

There were several overlapping between MHC class I (B-F) epitopes and MHC class II (B-L) epitopes. These overlapping are illustrated in Table (4).

Table.3 list of T Helper cell epitopes that had binding affinity with the Human MHC Class II alleles instead of B-L alleles

Epitope(Core)	Allele	percentile rank	smm_align_ic50	Peptide	Start	End
*YLALGVLRT	HLA-DRB1*11:01	0.34	207	SYQYLALGVLRTSAT	202	216
	HLA-DRB1*04:01	1.79	155			
	HLA-DPA1*03:01/DPB1*04:02	2.85	247			
	HLA-DRB1*01:01	3.97	8			
	HLA-DRB1*04:05	4.56	311			
	HLA-DRB1*15:01	9.01	465			
	HLA-DRB1*13:02	1.81	73	YLALGVLRTSATGRV	205	219
*LRGVFGTML	HLA-DRB1*07:01	1.08	39	YRNHTLRGVFGTMLD	479	493
	HLA-DRB5*01:01	3.44	410			
	HLA-DRB1*11:01	3.87	1070			
	HLA-DRB1*08:02	4.78	949			
	HLA-DRB1*04:05	4.84	329			
	HLA-DRB1*09:01	6.17	246			
	HLA-DRB1*01:01	7.34	29			
	HLA-DRB1*15:01	8.01	415			
	HLA-DRB1*03:01	9.51	487	LRGVFGTMLDGEQAR	484	498
**YTTSTCFKV	HLA-DRB1*09:01	1.86	276	YTTSTCFKVVKTNKT	526	540
	HLA-DRB5*01:01	3.44	35			
	HLA-DRB1*07:01	5.79	29			
	HLA-DPA1*01/DPB1*04:01	5.54	992	SIKAAYTTSTCFKVV	521	535
	HLA-DPA1*02:01/DPB1*05:01	9.61	1742	KAAYTTSTCFKVVKT	523	537
NRKSCSVSA	HLA-DRB1*07:01	2.05	181	DTQNRKSCSVSATPL	231	245
LESPLALLN	HLA-DRB1*04:01	2.79	134	VALESPLALLNTETT	88	102
	HLA-DPA1*03:01/DPB1*04:02	8.95	440			
	HLA-DRB5*01:01	9.43	775			
ALESPLALL	HLA-DQA1*05:01/DQB1*02:01	6.75	2073	YKQVALESPLALLNT	85	99
	HLA-DPA1*03:01/DPB1*04:02	6.76	232			
	HLA-DPA1*02:01/DPB1*01:01	8.66	259			
**VALESPLAL	HLA-DRB1*03:01	5.69	525	RIYKQVALESPLALL	83	97
	HLA-DRB5*01:01	9.43	772			
	HLA-DPA1*03:01/DPB1*04:02	9.48	246			
	HLA-DRB1*04:01	6.27	574	KQVALESPLALLNTE	86	100
*-**ISNTLFGEF	HLA-DPA1*01:03/DPB1*02:01	2.38	178	SIAEISNTLFGEFRI	544	558
	HLA-DPA1*01/DPB1*04:01	2.74	399			
	HLA-DPA1*02:01/DPB1*01:01	4.36	429			
	HLA-DRB3*01:01	6.08	7081			
	HLA-DPA1*02:01/DPB1*05:01	9.32	1266			
	HLA-DPA1*03:01/DPB1*04:02	8.31	998	AEISNTLFGEFRIVP	546	560
NHTLRGVFG	HLA-DRB1*08:02	3.25	976	FYRNHTLRGVFGTML	478	492
	HLA-DRB1*15:01	7.82	406			
QYLALGVLR	HLA-DRB5*01:01	3.73	442	SYQYLALGVLRTSAT	202	216
	HLA-DRB1*03:01	7.21	2308	QYLALGVLRTSATGR	204	218
TTSTCFKVV	HLA-DPA1*01/DPB1*04:01	5.51	988	IKAAYTTSTCFKVVK	522	536
EISNTLFGE	HLA-DPA1*01:03/DPB1*02:01	2.59	187	CLSIAEISNTLFGEF	542	556
	HLA-DPA1*01/DPB1*04:01	3.93	416			
	HLA-DPA1*02:01/DPB1*01:01	4.8	447			

Percentile rank ≤ 10 *suggested Epitopes ** CTL epitopes

Table.4 Overlapping between MHC class I and II T cell epitopes

CTL epitopes	sequence	position	T helper epitopes
AEISNTLFG	SI**AEISNTLFG**EFRI	554-558	*ISNTLFGEF
	AEISNTLFGEFRIVP	546-560	
	CLSI**AEISNTLFG**EF	542-556	EISNTLFGE
ISNTLFGEF	CLSIAE**ISNTLFGEF**	542-556	EISNTLFGE
YTTSTCFKV	IKAA**YTTSTCFK**VVK	522-536	TTSTCFKVV
VALESPLALL	**VALESPLAL**LNTETT	88-102	LESPLALLN
	YKQ**VALESPLAL**LNT	85-99	ALESPLALL

The underlined and highlighted residues are the 9-mer MHC class I T cell epitopes overlapping the 15-mer MHC class II T cell epitopes. * CTL epitopes

Table.5 B- cell epitopes predicted by different B cell epitope prediction tools

Epitope	start	End	length	Emini Surface Score/Threshold 1.000	Antigenicity Score/Threshold 1.034	Hydrophilicity Score/Threshold 1.549	Beta Turn Score/Threshold 1.008
PAPTTG	164	169	6	1.171	0.981	3.733	1.197
*QNRKSCSV	233	240	8	1.153	1.052	4.2	1.131
YPG	299	301	3	1.163	1.033	1.967	1.407
*SSY	418	420	3	1.365	1.062	3.7	1.333
DG	493	494	2	1.019	0.87	7.85	1.51

*proposed Epitopes

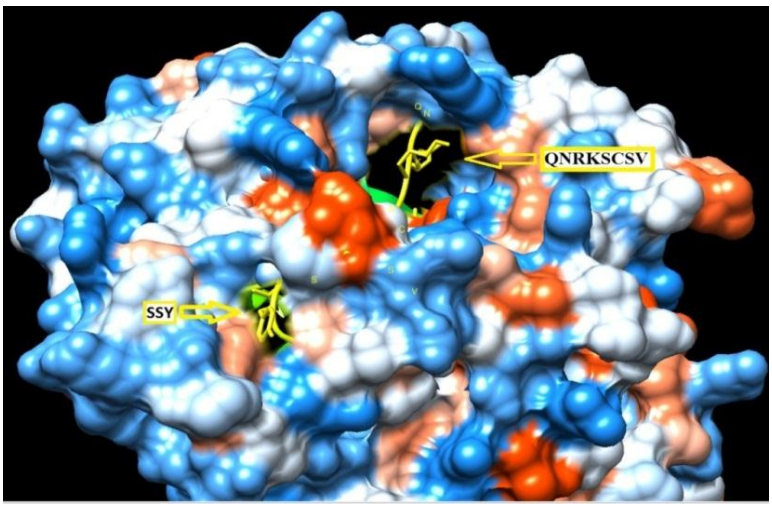

Figure.2 3D structure of Proposed B cell epitopes of HN protein in virulent strains of NDV virus illustrated by UCSF-Chimera visualization tool

3.4 B cell epitope Prediction and Modeling

Amino acid sequences of HN protein were subjected to Bepipred linear epitope prediction, Emini surface accessibility, Kolaskar and Tongaonkar antigenicity, Parker hydrophobicity and Chou and Fasman beta turn prediction tools in IEDB.

Five conserved B cell epitopes were predicted by B cell epitope prediction tools. Among these five epitopes; only two were found to satisfy all B cell tools: ^{233}**QNRKSCSV**^{240} and ^{418}**SSY**^{420} .The other three epitopes did not succeed the Kolaskar and Tongaonkar Antigenicity prediction tool. The result is summarized in Table (5), Figure (2) at the structural level.

3.5 Molecular Docking of B-F alleles and predicted CTL Epitopes

The four predicted CTL peptides that interacted with selected human's MHC-1 alleles: ^{548}**ISNTLFGEF**^{556}, ^{546}**AEISNTLFG**^{554}, ^{526}**YTTSTCFKV**^{534} and ^{88}**VALESPLAL**^{96} were used as ligands to detect their interaction with selected B-F alleles /receptors (BF2*2101, BF2*0401), Figure (3) by docking Techniques using off- and on-line softwares. Based on polar contacts and the binding energy in kcal/mol unit, the lowest binding energy (kcal/mol) was selected to obtain best binding (pose) and to predict real CTL epitopes as possible, Table (6), Figures (4 &5). The results were compared with positive control experiment (-8.72 kcal/mol for BF2*2101) and (-8.66 kcal/mol for BF2*0401), Table (7).

Table.6 Molecular docking results of predicted CTL epitopes (ligands)/ BF alleles (receptors)

Target/Receptor	Ligand	Binding Affinity Energy [a] (Kcal/mol)	Binding Free Energy [b] (Kcal/mol)	Scoring [c]	Number of polar contact of ligands
BF2*2101	A1: AEISNTLFG	-9.1	-8. 67	7624	7
	B1: ISNTLFGEF	-8.8	-8. 72	8202	6
	C1: YTTSTCFKV	-8.8	-8.71	8050	7
	D1: VALESPLAL	-7.2	-8. 63	8246	4
BF2*0401	A2: AEISNTLFG	-8.9	-8. 72	7994	12
	B2: ISNTLFGEF	-9.7	-8. 70	8324	12
	C2: YTTSTCFKV	-8.6	-8. 69	8276	11
	D2: VALESPLAL	-8.7	-8. 72	8288	7

[a] PyRx software [b] MTiAUTODOCK software [c] PatchDock software

Table.7 Molecular docking results of peptides interaction with BF2*2101 & BF2*0401 where used as positive control [44]

	BF2*2101				BF2*0401			
Ligand	PyRx-docking (Kcal/mole)	MTiAutodock (Kcal/mole)	PatchDock (score)	Polar contact	PyRx-docking (Kcal/mole)	MTiAutodock (Kcal/mole)	PatchDock (score)	Polar contact
*TPYDINQML	-8.2	-8.72	8226	12	-8.9	-8.66	7974	11
QYDDAVYKL	-8.8	-8.71	7598	8	-9.6	-8.68	7262	15
NPRAMQALL	-8.4	-8.71	7934	5	-8.2	-8.74	7574	10
VMAPRTVLL	-7.7	-8.7	8008	2	-8.9	-8.71	8314	9
RIIPRHLQL	-8.1	-8.7	7824	13	-8.8	-7.21	7824	15
GILGFVFTL	-8.5	-8.69	8908	11	-9.1	-8.66	9028	11
EEPTVIKKY	-8.6	-8.68	8326	13	-8.6	-8.71	8354	9
RRKWRRWHL	-8.9	-8.56	8710	15	-8.0	-8.41	8480	12

*selected for positive control

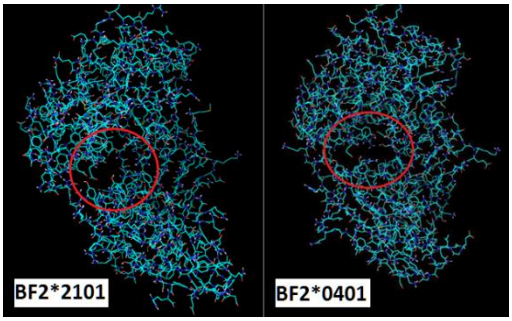

Figure.3 shows the Receptors in cartoon lines representation with active site indicated as red circle illustrated by PyMOL software

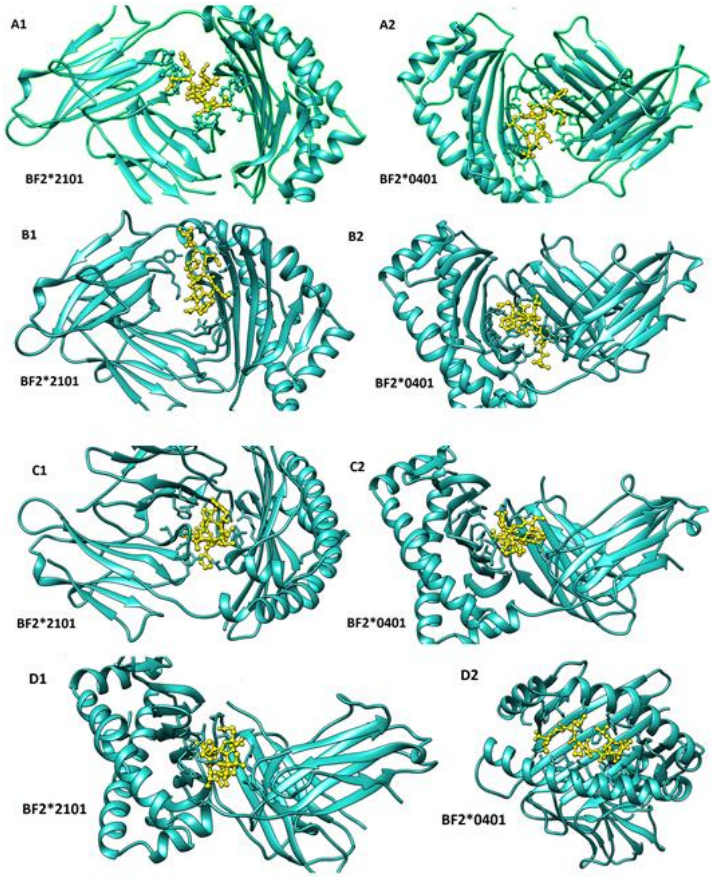

Figure.4 Pictures show the interaction between epitopes and receptors using UCSF-Chimera visualization tool after online docking. Receptors (BF alleles) represent by green colour while CTL Epitopes represent by yellow one. **A:** AEISNTLFG, **B:** ISNTLFGEF **C:** YTTSTCFKV, **D:** VALESPLAL

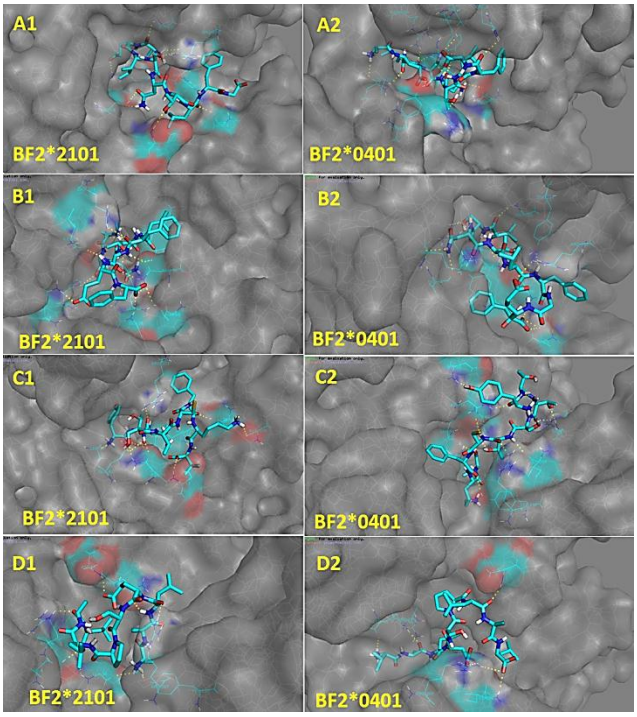

Figure.5 PyMOL visualization shows the polar binding interaction between epitopes and receptors after docking. By PyRx software Epitopes coloured by **CHNOS.** While polar contacts appear in yellow one. **A:** AEISNTLFG, **B:** ISNTLFGEF **C:** YTTSTCFKV, **D:** VALESPLAL

4. Discussion

Vaccination is generally considered as the most effective method of preventing infectious diseases. All vaccinations work is done by presenting a foreign antigen to the immune system in order to evoke an immune response [53]. So, we aimed to design a peptide vaccine for NDV particularly for the haemagglutinin neuraminidase protein (HN) using computational methods to predict the immune system inducing epitopes.

We all know that, CD8+ cytotoxic T lymphocytes (CTLs) plays a crucial role in fighting viral infections after they interact with MHC-I alleles [36]. MHC class-I alleles (HLA-A and -B in man) were chosen in this study instead of B-F alleles due to the non-availability of the B-F alleles by epitope prediction softwares and due to similarity between MHC Class I and B-F biochemically and functionally in antigen presentation of T lymphocyte and their stimulation of immune system to mammalian Class I homologs [54]. According to Table (2); we found that four CTLs' conserved epitopes: ^{548}**ISNTLFGEF**^{556} interacted with two human's alleles: HLA-B*44:02 and HLA-B*15:01. The other peptides ^{546}**AEISNTLFG**^{554}, ^{526}**YTTSTCFKV**^{534} and ^{88}**VALESPLAL**^{96} interacted with HLA-B*44:02, HLA-A*68:02 and HLA-B*46:01 respectively. These CTL epitopes docked and interacted with BF2*2101, BF2*0401 trying to suggest presence of real CTL epitopes. We selected these two alleles based on two studies; the first was done by Kokh M. *et al.* (2007) and reported the presence of the first structures of an MHC molecule (BF2*2101) in chicken MHC haplotype B21, not in mammals. This molecule has a novel mode of peptide binding that allows peptides with completely different sequences [44] and unusually large peptide-binding groove that accommodates a broad spectrum of peptides that were present as epitopes to CTL [44]. The second Study was done by Zhang J. *et al.* (2012) and reported the crystal structure of BF2*0401 from the B4 (also known as B13) haplotype. This allele was highly positively charged and this property limits the number of epitope peptides that can bind this class I molecule. However, peptide-binding assays show that in vitro, BF2*0401 can bind a wider variety of peptides than the ones found on the surface of B4 cells [45]. We did some processing in BF2*2101/receptor (PDB ID: 3BEW which include removing of a ligand). We tried to find true epitopes based on their docking properties (completely located in the groove and the number of polar contacts) by comparing them with positive control. We madethis positive control after docking the eight peptides used by Kokh M. *et al.* (2007) [44] with selected B-F alleles (BF2*2101 and BF2*0401) using the whole structure of these alleles. Then we selected the lowest binding free energy scores (ranked highest) namely **TPYDINQML** and used as positive control which achieved binding free energy (-8.72 and -8.66 Kcal/mole) with (BF2*2101 and BF2*0401), respectively. After comparing our findings with positive control we made; we found the following; in BF2*2101 receptor the CTL epitope (^{548}**ISNTLFGEF**^{556}) had score equal to positive control and other three CTL epitopes around this energy binding. While in BF2*0401 receptor all our predicted CTL epitopes achieved energy score above the positive control. In addition, BF2*0401 receptor had binding affinity greater than the affinity found in BF2*2101 receptor based on polar contact (H-bond) with the CTL epitopes/Ligands. These polar contacts were equal to or near to positive controls (especially in BF2*0401 receptor). Moreover; we observed that the active sides in receptor (BF2*0401) contains amino acids with polar part as numerous arginine and aspartate residues, in addition to polar atoms like serine and sparagine which facilitate polar interaction and strength the binding. Also we found ^{88}**VALESPLAL**^{96} epitope had additional binding site with BF2*0401 receptor.

Depending on this fact "Helper T cells are arguably the most important cells in adaptive immunity, as they are required for almost all adaptive immune responses. Not only they help activate B cells to secrete antibodies and macrophages to destroy ingested microbes, but they also help activate cytotoxic T cells to kill infected target cells" [55] Activation of B cell required interaction with MHC II alleles [7] . We depended on human MHC class II allele (HLA DR, DP and DQ) due to difficulty to determine avian B-L alleles and due to Serological, structural and functional studies of B-L antigens revealed a great similarity with la antigens of mammals. Similar B-L antigens were expressed in the cell membrane of Ig positive lymphocytes, monocytes/macrophages and some stimulated T cells [56]. According to Table (3); we found 12 conserved T helper epitopes interacted with different set of human's alleles of (HLA-DRB1, HLA-DRB3, HLA-DRB5, HLA-DPA1/HLA-DPB1 and DQA1/ DQB1).Among these 12

epitopes; we found epitopes **[484]LRGVFGTML[492]** and **[205]YLALGVLRT[213]** had the affinity to interact with each of the 9 and 7 alleles**,** respectively**.** While **[548]ISNTLFGEF[556]** epitope had the affinity to interact with of the 11 alleles, but we did not find B-L alleles to test these two highest binding affinity epitopes **[484]LRGVFGTML[492]** and **[205]YLALGVLRT[213]** using the docking technique. Beside the CTL epitope **[548]ISNTLFGEF[556]** we found other promising CTL epitopes **[526]YTTSTCFKV[534]** and **[88]VALESPLAL[96]** had ability to bind with seven and five Human MHC class II alleles, respectively and could activate both T lymphocytes (CTL and HTL) in cellular immunity.

Chen F *et al*. (2012) compared the 3-D structure of chicken B-L with human HLA-DR1 molecules and demonstrated their structural similarities. They found that the percentage of homology between both molecules was only 66%. Some conserved sites in human and chicken MHC class II molecules reflected their common ancestry and similar functions and they found the chicken B-L molecule had more polymorphic sites than the human HLA-DR1 molecule, which presumably might be a mechanism to compensate for responding to a wider array of pathogens due to fewer loci for chicken [57]. According to this similarity between HLA-DR/ B-L molecules we observed that HLA-DRB1 alleles were the most common ones that interacted with 12 predicted epitopes with the percentile rank <10 which increase the possibility of our predicted peptides to be true ones.

A study done by Béhar G. *et al.* (1998) used a probe derived from an HLA-DQ β cDNA clone by cross-hybridization. Exons encoding the β1, β2 and transmembrane domains of a B-L β chain were identified with 63, 66 and 62% similarity with the HLA-DQ β sequence. This first isolation of an MHC class II gene outside of the mammalian class provides an insight into the evolution of MHC genes based on the comparison of avian and mammalian class II β chain amino acid and nucleotide sequences [58]. But we found that only one of HLA-DQ β allele (DQB1*02:01) interacted with the **[89]ALESPLALL[9]** epitope within our selected percentile rank ≤ 10 (6.75).

In our study; according to Table (4) we observed several overlapping peptides/epitopes, suggesting the possibility of antigen presentation to immune cells via both MHC class I (B-F) and II (B-L) pathways especially the overlapping between **[548]ISNTLFGEF[556]** and **[546]AEISNTLFG[554]**.

When the B cell epitope scores (or antibody Epitopes) appeared above the threshold in four algorithms of Bepipred linear epitope prediction, Emini surface accessibility, Parker hydrophobicity, Kolaskar and Tongaonkar antigenicity in IEDB; that means these candidate epitopes could be potential and effective peptide antigens for B cell. These threshold scores were used as the default setting to avoid variation of our results. The Chou and Fasman beta turn prediction method was used also in this study because the antigenic parts of a protein belong to the beta turn regions [59]. Based on Table (5); we found only two epitopes **[233]QNRKSCSV[240]** and **[418]SSY[420]** satisfied these default threshold scores and may immunity. The limitation of this study appears in using the MHC class-I alleles (HLA-A and -B in man) instead B-F alleles and MHC class II allele (HLA DR, DP and DQ in man in chickens) instead B-L alleles.

In summery; from best predicted highly conserved B and T epitopes. We suggested that, the 9-mer CTL epitopes (**[548]ISNTLFGEF[556], [546]AEISNTLFG[554], [88]VALESPLAL[96]** and **[526]YTTSTCFKV[534]**) were able to interact with MHC class I alleles (B-F alleles) and could become universal peptide based vaccine against NDV. We found these CTL epitopes to be T helper epitopes. The overlapping between MHC Class I (B-F) and II (B-L) T cell epitopes suggested the possibility of antigen presentation to immune cells via both MHC class I and II pathways especially the overlapping between **[548]ISNTLFGEF[556] and [546]AEISNTLFG[554].**

Conclusion

Our results are based on the predictive and analytic tool (IEDB-AR) and can provide a comprehensive analysis resource that can help researchers to develop prophylactic\therapeutic methods against new and old diseases. This immunoinformatics tool used in a variety of applications from basic immunological data to computational techniques and assays to deliver effective biomedical research for prediction of new epitopes , vaccines design and design of immune-based cancer therapies. Based on this protocol; the B-cell epitopes, helper T-cell epitopes and CTL epitopes were predicted from the HN protein of virulent strains of NDV. Then CTL epitopes were selected as vaccine candidates. We can confirm our findings by adding complementary steps of both in vitro and in vivo studies to support this new universal predicted vaccine for chicken.

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Competing Interests

The authors declare that they have no competing interests.

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