Vaccine Design against Coronavirus Spike (S) Glycoprotein in Chicken: Immunoinformatic and Computational Approaches

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Abstract

Background

Infectious bronchitis (IB) is a highly contagious respiratory disease in chickens and produces economic loss within the poultry industry. It is caused by a single stranded RNA virus belonging to Cronaviridae family.

Methods

The present study used various tools in Immune Epitope Database (IEDB) to predict conserved B and T cell epitopes against IBV spike (S) protein that may perform a significant role in provoking the resistance response to IBV infection. Structural analysis, homology modelling and molecular docking were also achieved.

Results

In B cell prediction methods, three epitopes (\text{1139KKSSYY}_{1144}, \text{1140KSSYYT}_{1145}, \text{1141SSYYT}_{1145}) were selected as surface, linear and antigenic epitopes based on the length and antigenicity score. Many MHCI and MHCII epitopes were predicted for IBV S protein. Among them \text{982YYITARDMY}_{990} and \text{983YITARDMYM}_{991} epitopes displayed high antigenicity, no allergenicity and no toxicity as well as great linkage with MHCI and MHCII alleles. Moreover, docking analysis of MHCI epitope produced strong binding affinity with BF\textsubscript{2} alleles.

**Conclusion:** Five conserved epitopes were expected from spike glycoprotein of IBV as the top B cell and T cell epitopes due to high antigenicity, no allergenicity and no toxicity. In addition, MHC epitopes showed great linkage with MHC alleles as well as strong interaction with BF2 alleles. These epitopes should be designed and incorporated and then tested as multi-epitope vaccine against IBV.

Backgrounds

Infectious bronchitis virus (IBV) is a single positive stranded RNA that belonging to coronavirus of the chicken (\textit{Gallus gallus}). It is a highly contagious respiratory disease in chickens that is mainly severe for very young chicks. The signs of illness include tracheal rales, coughing, sneezing, nasal discharge and some strains may cause kidney damage [1, 2]. The disease can be transmitted by infected
chickens in respiratory discharges and feces, and it can be spread by aerosol, ingestion of contaminated feed and water, and contact with contaminated equipment or clothing. The virus cannot be transmitted via eggs [3]. The disease causes economic loss within the poultry industry, affecting the performance of meat-type and egg-laying birds. The disease can affect all ages, but the clinical disease is more severe in young chicks. Chicks become more resistant to IBV-induced mortality with increasing age [4].

There are four structural proteins associated with the envelope, the spike (S), membrane (M), envelope (E), and nucleocapsid (N) protein [5]. The spike ‘S’ glycoprotein is located at the surface of the virion and consists of two subunits, SI and S2. The membrane ‘M’ glycoprotein is partially exposed at the surface of the virion and the nucleocapsid ‘N’ protein that located internally. The spike glycoprotein of IBV induces virus neutralizing (VN) and HI antibodies and has been considered as the most likely inducer of protection [2, 4]. The spike S protein is a dimer or trimer. It has two known functions; to attach the virus to receptor molecules on host cells, and to activate fusion of the virion membrane with host cell membranes, to release the viral genome into the cell [2]. The spike gene is highly variable, especially the S1 part, due to insertions, deletions, substitutions and recombination events [6]. Application of vaccine is the most effective way of protective against pathogenic, specifically when these pathogens have a high mortality rate such as IBV and virus in general. On the other hand, the large number of serotypes and strains (genotype) of IBV make control process complicated precisely. IBV has shift and drift property [7].

Vaccination with inactivated vaccines and live-attenuated vaccines is used to control the disease. However, inactivated vaccines frequently fail to induce strong cellular immunity, while live-attenuated vaccines can contribute to the emergence of antigenic variant viruses [5]. The increasing number of new serotypes of IBV, which were caused by frequent gene mutation and recombination, are a major challenge for the prevention and control of IB disease [8].

Moreover, RNA viruses have high mutational rates, such as IBV. So the most important step in the design of cross-protective peptide vaccine against IBV is to target the conserved epitopes of different strains of IBV [5].
There is an essential need for the development of safer and more effective vaccines to control IBV. Therefore, the aim of this study is to analyze strains of spike (S) glycoprotein of infectious bronchitis virus reported in NCBI database using immunoinformatics and computational approaches to select all possible epitopes that can be used as multi-epitopes vaccine.

2. Methods
2.1. Protein Sequence Retrieval
Spike (S) protein sequences of different infectious bronchitis virus (IBV) strains were retrieved from the GeneBank of National Central Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/protein/) database in March 2019. The sequences were saved in FASTA format (Table 1).

2.2 Structural Analysis
Reference sequence of spike S protein (NP_040831.1) was analyzed to identify the chemicals and physical properties including GRAVY (Grand average of hydropathicity), half-life, molecular weight, stability index and amino acid atomic composition using an online tool Protparam [9].

Secondary structure of spike S protein of IBV was analyzed through PSIPRED [10]. The secondary structure of protein including helix, sheet, turn, and coil parameters was predicted using GOR IV server at https://npsa-prabi.ibcp.fr/cgi-bin/secpred_sopma.pl.

TMHMM an online tool (http://www.cbs.dtu.dk/services/TMHMM/), used to examine the trans-membrane topology of S protein. Presence of disulphide-bonds were predicted through an online tool DIANNA v1.1. It makes prediction based on trained neural system [11]. CDD-BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) [12-14] and PFAM (http://www.pfam.sanger.ac.uk/) 229E [15] were used to search the defined conserved domains in the targeted protein sequences.
Table 1
Accession numbers, date and area of collection of the retrieved sequences of Spike protein sequences of IBV.

| No | Accession No | Country | Year | No | Accession No | Country | Year |
|----|--------------|---------|------|----|--------------|---------|------|
| 1  | NP_040831.1  | UK      | 2018 | 47 | AAV98206.1   | USA     | 2002 |
| 2  | AHX25911.1   | China   | 2016 | 48 | AVX27612.1   | India   | 2004 |
| 3  | AHX25902.1   | China   | 2016 | 49 | ALE71331.1   | India   | 2018 |
| 4  | AHX25893.1   | China   | 2016 | 50 | AJP16712.1   | China   | 2015 |
| 5  | AMK51938.1   | China   | 2016 | 51 | AJP16739.1   | China   | 2015 |
| 6  | AEPB4746.1   | China   | 2016 | 52 | AFP50306.1   | Korea   | 2015 |
| 7  | AEPB4736.1   | China   | 2016 | 53 | AFP50302.1   | Korea   | 2012 |
| 8  | ACX71849.1   | China   | 2011 | 54 | AFP50294.1   | Korea   | 2012 |
| 9  | ACX71844.1   | China   | 2011 | 55 | AEP50274.1   | Korea   | 2012 |
| 10 | ACX71842.1   | China   | 2011 | 56 | AEL12221.1   | China   | 2012 |
| 11 | AAO09490.1   | China   | 2011 | 57 | ADY62552.1   | China   | 2012 |
| 12 | AAY24433.1   | Singapore | 2005 | 58 | ADV71785.1   | Netherlands | 2010 |
| 13 | AAY24423.1   | Singapore | 2005 | 59 | ACQ55230.1   | Netherlands | 2011 |
| 14 | AAY21248.1   | Singapore | 2005 | 60 | AEE67884.1   | Pakistan | 2017 |
| 15 | AAY21247.1   | Singapore | 2005 | 61 | ARB66180.1   | China   | 2017 |
| 16 | AAY21246.1   | Singapore | 2005 | 62 | AQQ55821.1   | China   | 2017 |
| 17 | AAY21245.1   | Singapore | 2005 | 63 | AHX26172.1   | China   | 2016 |
| 18 | AAY21244.1   | Singapore | 2005 | 64 | AHX26163.1   | China   | 2016 |
| 19 | AAY21243.1   | Singapore | 2005 | 65 | AHX26154.1   | China   | 2016 |
| 20 | AAY21242.1   | Singapore | 2005 | 66 | AHX26145.1   | China   | 2016 |
| 21 | AGW24533.1   | India   | 2015 | 67 | AHX26136.1   | China   | 2016 |
| 22 | AAW33786.1   | USA     | 2006 | 68 | AHX26127.1   | China   | 2016 |
| 23 | AER08740.1   | Sweden  | 2012 | 69 | AHX26118.1   | China   | 2016 |
| 24 | AER08739.1   | Sweden  | 2012 | 70 | AHX26109.1   | China   | 2016 |
| 25 | AER08729.1   | Sweden  | 2012 | 71 | AHX26073.1   | China   | 2016 |
| 26 | AER08728.1   | Sweden  | 2012 | 72 | AHX26064.1   | China   | 2016 |
| 27 | AER08727.1   | Sweden  | 2012 | 73 | AHX26055.1   | China   | 2016 |
| 28 | AER08726.1   | Sweden  | 2012 | 74 | AHX26046.1   | China   | 2016 |
| 29 | AER08725.1   | Sweden  | 2012 | 75 | AHX26037.1   | China   | 2016 |
| 30 | AER08724.1   | Sweden  | 2012 | 76 | AHX26028.1   | China   | 2016 |
| 31 | AER08723.1   | Sweden  | 2012 | 77 | AHX26019.1   | China   | 2016 |
| 32 | AER08722.1   | Sweden  | 2012 | 78 | AHX26010.1   | China   | 2016 |
| 33 | AER08721.1   | Sweden  | 2012 | 79 | AHX26001.1   | China   | 2016 |
| 34 | ADA83557.1   | USA     | 2011 | 80 | AHX25992.1   | China   | 2016 |
| 35 | ADA83467.1   | USA     | 2011 | 81 | AHX25983.1   | China   | 2016 |
| 36 | ABHO1142.1   | USA     | 2007 | 82 | AHX25974.1   | China   | 2016 |
| 37 | ABHO1141.1   | USA     | 2007 | 83 | AHX25965.1   | China   | 2016 |
| 38 | ABI26423.1   | USA     | 2006 | 84 | AHX25966.1   | China   | 2016 |
| 39 | AAK27168.1   | China   | 2005 | 85 | AHX25947.1   | China   | 2016 |
| 40 | ACH72794.1   | China   | 2009 | 86 | AHX25938.1   | China   | 2016 |
| 41 | AAW83034.1   | China   | 2006 | 87 | AHX25929.1   | China   | 2016 |

2.3 Multiple Sequence Alignment And Epitope Conservancy Assessment
The retrieved sequences of IBV S protein were aligned using Clustal program and consensus sequence was generated using the multiple sequence alignment (MSA) tool, Jalview version 2.10.5. (http://www.jalview.org/about/jalview-scientific-advisory-committee) [16]. Epitope conservancy
analysis in Immune Epitope Database (IEDB) was used to detect potential epitope conservancy (http://tools.iedb.org/conservancy/) [17]. For calculating the conservancy score, the sequence identity threshold was kept at 80%.

2.4 Phylogeny Analysis
Phylogenetic tree of the retrieved sequences of spike (S) protein was performed using MEGA7.0.26 (7170509) software using maximum likelihood parameter [18].

2.5 B Cell Prediction
The Immune Epitope Database (IEDB) (http://tools.iedb.org/mhci/) was used to predict B and T cell epitopes of IBV reference sequence of S proteins (NP_040831.1) [19]. Linear B-cell epitopes were predicted using BepiPred from IEDB [20]. Emini surface accessibility prediction tool was used to predict epitopes located on the surface [21]. Whereas, the antigenic epitopes were investigated using kolaskar and Tongaonkar antigenicity method [22].

2.6 T-cell Epitope Prediction
The T cell epitopes were predicted in human among different alleles of major histocompatibility complex class I (MHCI) and class II (MHCII). MHCI binding epitopes were predicted using artificial neural networks (ANN) [23, 24]. Peptide length was set as 9 amino acids. The half maximal inhibitory concentration (IC50) values of the peptides binding to MHCI molecule was calculated and the epitope that had IC50 with binding affinity less or equal to 300 nm were suggested as promising candidate epitopes. MHC class II molecules was performed by the IEDB MHCII prediction tool at (http://tools.iedb.org/mhcii/) [19]. Human MHC class II alleles (HLA DR, HLA DP and HLA DQ) were used for MHCII binding predication. NN-align method was used with IC50 less or equal to 1000 nM [25].

2.7 Antigenicity, Allergenicity And Toxicity Of Epitopes
Vaxijen v2.0 server (http://www.ddg-pharmfac.net/vaxijen/Vaxijen/Vaxijen.html) was used to predict the antigenicity of the conserved regions [26]. It was used with the default prediction parameters and a threshold value of 0.4. The in silico allergenicity prediction of epitopes was investigated using AllerTop v .2.0 (http://www.ddg-pharmfac.net/AllerTOP) [27]. While ToxiPred server was used to predict the toxicity of predicted epitopes. (http://crdd.osdd.net/raghava/toxinpred/) [28].

2.8 Homology Modeling:
IBV reference sequences was submitted to SWISS-MODEL (http://swiss-model.expasy.org/) for protein structure homology modelling [29–33]. The protein sequences of BF alleles (BF2 *2101 and BF2*0401) were submitted to Raptor X server (http://raptorx.uchicago.edu/) to design their three D structures [34–36]. Chimera software 1.8 was used to visualize the 3D structures of the IBV reference sequences and BF alleles [37].

2.9 Molecular Docking
To perform molecular docking, 3D structure of MHCI epitopes and 3D modeled of both BF alleles were submitted simultaneously to PatchDock online autodock tools; an automatic server for molecular docking (https://bioinfo3d.cs.tau.ac.il/PatchDock/) [38]. Firedock (http://bioinfo3d.cs.tau.ac.il/FireDock/) was used to select the five top models [38]. Visualization of the result was performed using UCSF-Chimera software 1.8 [37].

3. Results
3.1 Structural analysis
The physiochemical properties of spike S protein calculated by protparam revealed that it contained 1162 amino acids (aa) with molecular weight of 128046.70 kDa, which reflects good antigenic nature. Theoretical isoelectric point (PI) of subject protein was 7.71 which indicate its positive in nature. An isoelectric point above 7 shows positively charged protein. Approximately, 81 aa were found as negatively charged whereas 84 aa found as positively charged. Protparam computed instability-index (II) 35.53, this categories protein as stable. Aliphatic-index 86.05, which devotes a thought of proportional volume hold by aliphatic side chain and GRAVY value for protein sequence is 0.012. Half-life of protein depicted as the total time taken for its vanishing after it has been synthesized in cell, which was computed as 30 h for mammalian-reticulocytes, > 20 h for yeast, > 10 h for Escherichia coli. Total number of Carbon (C), Oxygen (O), Nitrogen (N), Hydrogen (H) and Sulfur (S) were entitled by Formula: C$_{5737}$H$_{8847}$N$_{1495}$O$_{1718}$S$_{56}$. Secondary structure of spike S protein of IBV was analyzed through PSIPRED and GOR IV server. The component of secondary structure prediction by GOR IV server are alpha helix (29.43%), extended strand (27.37%), beta turn (5.25%), and random coil (37.95%) (Fig. 1). DiANNA1.1 tool calculated 19 disulphides bonds (S–S) positions and assign them a score and it makes prediction based on trained neural system. The trans-membrane protein topology
was checked via online tool TMHMM and it was found that residue from 1 to 1093 were exposed on
the surface, while residue from 1094 to 1116 were inside trans-membrane-region and residues from
1117 to 1162 were buried within the core-region of the S protein (Fig. 1).
Two conserved domains (Corona-S1 and Corona-S2) were identified in refseq of IBV spike
glycoprotein. The conserved domains were sequenced by Conserved Domain (CDD) BLAST search. It
stated that corona-S1 (pfam01600) is the only member of the superfamily cl03276 and corona-S2
domain (pfam01601) is the only member of the superfamily cl20218. The top related sequences in
both domains were Feline infectious peritonitis virus (strain 79-1146), Avian infectious bronchitis virus
(strain Beaudette), and Human coronavirus 229E while Severe acute respiratory syndrome-related
coronavirus sequences was only associated to corona-S2 domain [39].
3.2 Multiple Sequence Alignment
Jalview was used to visualize the multiple sequence alignment of the retrieved sequences. In
alignment, several areas have been shown to have mutation see Fig. 2.
3.3 Phylogeny
Phylogenetic trees for targeted proteins were constricted using MEGA7.0.26 (7170509) software using
maximum likelihood parameter see Fig. 3.
3.4 B-cell Epitopes
In B cell prediction methods, several epitopes using Bepipred Linear Epitope Prediction method were
predicted. The conservancy percentages of these epitopes were presented in Table 2 and 3. Twenty
one linear conserved epitopes were identified after shortened of predicted epitopes. Of these, seven
epitopes with different length between the positions 1139-1146 were identified as linear, surface and
antigenic epitopes (see Table 3). These epitopes were $^{1139}{\text{KKSSYY}}_{1144}, ^{1140}{\text{KSSYYT}}_{1145},$
$^{1141}{\text{SSYYTT}}_{1146}, ^{1141}{\text{SSYYT}}_{1145}, ^{1142}{\text{SYTT}}_{1146}, ^{1142}{\text{SYTT}}_{1145}, \text{ and } ^{1143}{\text{YYTT}}_{1146}$. Three epitopes
$^{1139}{\text{KKSSYY}}_{1144}, ^{1140}{\text{KSSYYT}}_{1145}, ^{1141}{\text{SSYYTT}}_{1146}$ were selected as top B cell epitopes based on the
length and antigenicity score.
Table 2
Conservancy Assessment of B cell linear epitopes

| Epitope no | Epitope sequence | Start | End | Epitope length | Percent of protein sequence matches at identity ≤ 100% |
|------------|------------------|-------|-----|----------------|--------------------------------------------------|
| 1          | MTAPSSGMAW       | 83    | 92  | 10             | 89.13% (82/92)                                   |
| 2          | GGPI             | 193   | 196 | 4              | 90.22% (83/92)                                   |
| 3          | TGNFSD           | 235   | 240 | 6              | 97.83% (90/92)                                   |
| 4          | GPLQGGCK         | 352   | 359 | 8              | 94.57% (87/92)                                   |
| 5          | DSIV             | 450   | 453 | 4              | 91.30% (84/92)                                   |
| 6          | VNETGSQ          | 512   | 518 | 7              | 96.74% (89/92)                                   |
| 7          | RNETGSQ          | 512   | 518 | 7              | 94.57% (87/92)                                   |
| 8          | VGQKE            | 642   | 646 | 5              | 81.52% (75/92)                                   |
| 9          | STPKAGFNTP       | 656   | 665 | 10             | 81.52% (75/92)                                   |
| 10         | PONAPN           | 926   | 931 | 6              | 98.91% (91/92)                                   |
| 11         | ANASQY           | 959   | 964 | 6              | 98.91% (91/92)                                   |
| 12         | IVPA             | 966   | 969 | 4              | 86.96% (80/92)                                   |
| 13         | DVDFN            | 1026  | 1030 | 5         | 84.78% (78/92)                                   |
| 14         | SKWWDNKHELP      | 1034  | 1045 | 12        | 94.57% (87/92)                                   |
| 15         | GKKSSYYTT        | 1138  | 1146 | 9            | 97.83% (90/92)                                   |

Table 3
List of shortened B cell epitopes that has high score in both Emini and kolaskar

| No. | Peptide | Start | End | Length | Emini | koleskar |
|-----|---------|-------|-----|--------|-------|----------|
| 1   | SSYYTT  | 1141  | 1146| 6      | 2.568 | 1.027    |
| 2   | SYYT    | 1142  | 1146| 5      | 2.359 | 1.03     |
| 3   | YYTT    | 1143  | 1146| 4      | 1.26  | 1.035    |
| 4   | KKSSYY  | 1139  | 1144| 6      | 4.931 | 1.034    |
| 5   | KSSYYT  | 1140  | 1145| 6      | 3.559 | 1.031    |
| 6   | SSYTT   | 1141  | 1145| 5      | 2.191 | 1.051    |
| 7   | SYYT*   | 1142  | 1145| 4      | 2.019 | 1.061    |

3.5 Prediction Of MHC Class I Epitopes

In this study, Human MHC class-I HLA alleles were used to explore the interaction of epitopes with MHCI alleles using epitope prediction softwares as a result of the nonexistence of chicken MHC alleles in IEDB database. MHC-1 binding prediction tool using IEDB database expected thirteen conserved epitopes of Spike protein (S) which were interacted with many alleles in cytotoxic T cell. These epitopes were: 1115FFMTGCCGC1123, 590FNLTVTDEY598, 734GLLVLPPI742, 1105IFILILGW1113, 1139KKSSYYTT1147, 1087KTYIKWPWY1095, 166SVYLNDLV174, 985TARDYMMPR993, 1145TTFDNDVVT1153, 983YITARDYM1991, 1144YTTFDNDV1152, 982YITARDMY990, 1143YTTFDNDV1151.

3.6 Prediction Of MHC Class II Epitopes

MHC-II binding prediction tool based on NN-align with half-maximal inhibitory concentration (IC50) ≤ 1000 was used. Thirty conserved core sequences were predicted to interact with MHCII alleles. These cores were: 694EDLLFTSVE702, 1147FDNDVTEQ1155, 1115FFMTGCCGC1123, 1116FMTGCCGCC1124.
3.7 Antigenicity, allergenicity and toxicity of MHCI and MHCII epitopes:

The predicted MHCI and MHCII epitopes were subjected to VaxiJen v2.0 server, AllerJen v2.0. and ToxiPred to predict the antigenicity, allergenicity and toxicity of predicted epitopes. Five MHCI epitopes were identified as antigenic, non-allergic and non-toxic, but only three epitopes showed high linkage with MHCI alleles which were \(985\) TARDMYMPR, \(983\) YITARDMYM, and \(982\) YYITARDMY (Table 6). While six MHCII epitopes were predicted as antigenic, non-allergic and non-toxic epitopes (Table 7). However, \(983\) YITARDMYM and \(982\) YYITARDMY epitopes which were also presented in MHCII prediction methods, showed high antigenicity, no allergenicity and no toxicity. These epitopes were interacted with 52 and 38 alleles in MHCII see Fig. 4.

| Peptide   | Start | End | Antigenicity | Allele              | IC50  |
|-----------|-------|-----|--------------|---------------------|-------|
| YYITARDMY*| 982   | 990 | 0.8845       | HLA-A*29:02         | 14.52 |
|           |       |     |              | HLA-A*30:02         | 160.94|
|           |       |     |              | HLA-C*14:02         | 27.32 |
| YITARDMYM*| 983   | 991 | 0.7901       | HLA-A*02:01         | 233.08|
|           |       |     |              | HLA-A*02:06         | 212.86|
|           |       |     |              | HLA-C*03:03         | 29    |
|           |       |     |              | HLA-C*06:02         | 200.39|
|           |       |     |              | HLA-C*07:01         | 267.22|
|           |       |     |              | HLA-C*14:02         | 49.52 |
|           |       |     |              | HLA-C*15:02         | 77.63 |
| TARDMYMPR*| 985   | 993 | 0.6914       | HLA-A*30:01         | 56.23 |
|           |       |     |              | HLA-A*31:01         | 14.3  |
|           |       |     |              | HLA-A*68:01         | 28.24 |
| IIFILILGW | 1105  | 1113| 0.6749       | HLA-B*57:01         | 78.45 |
|           |       |     |              | HLA-B*58:01         | 64.27 |
| KKSSYTYTF | 1139  | 1147| 1.1865       | HLA-A*32:01         | 182.52|

| Core Sequence | Antigenicity | Peptide Sequence | Start | End | Allele      | IC50  |
|---------------|--------------|------------------|-------|-----|-------------|-------|
| IIFILILGW     | 0.6914       | IAPATIIFILILGWV  | 1100  | 1114| HLA-DRB1*15:01 | 454.6 |
| Sequence     | HLA-DQA1 | HLA-DPB1 |
|--------------|----------|----------|
| KKSSYYTTF 0.6749 | HLA-DPA1*01:03/DPB1*02:01 | 872.7 |
|               | HLA-DPA1*01/DPB1*04:01 | 408.1 |
|               | HLA-DPA1*01:03/DPB1*02:01 | 301.5 |
|               | HLA-DPA1*02:01/DPB1*05:01 | 953.4 |
| KCGKKSSYYTTF 1136 | HLA-DPA1*01/DPB1*04:01 | 276.8 |
|               | HLA-DPA1*02:01/DPB1*05:01 | 853.9 |
|               | HLA-DPA1*01:03/DPB1*02:01 | 958.9 |
| KSSYYTTFD 0.6466 | HLA-DPA1*01:03/DPB1*02:01 | 872.7 |
|               | HLA-DPA1*01:03/DPB1*02:01 | 155 |
|               | HLA-DPA1*01:03/DPB1*02:01 | 125.6 |
|               | HLA-DPA1*01/DPB1*04:05 | 92.2 |
|               | HLA-DPA1*01/DPB1*04:05 | 51.9 |
|               | HLA-DPA1*01:03/DPB1*02:01 | 46.9 |
| TARDMYMPR 0.7901 | HLA-DRB1*01:01 | 269.3 |
| YITARDMYMPR 1.1865 | HLA-DRB1*01:01 | 22 |
|               | HLA-DRB1*01:01 | 145 |
|               | HLA-DRB1*01:01 | 331.2 |
|               | HLA-DRB1*01:01 | 20.3 |
|               | HLA-DRB3*01:01 | 550.7 |
|               | HLA-DRB5*01:01 | 227.8 |
| VNGSYYITARD 978 | HLA-DRB1*01:01 | 338.6 |
|                | HLA-DRB1*01:01 | 25.8 |
|                | HLA-DRB1*01:01 | 447.6 |
|                | HLA-DRB1*01:01 | 105.8 |
|                | HLA-DRB1*01:01 | 248.3 |
|                | HLA-DRB1*01:01 | 27.8 |
|                | HLA-DRB1*01:01 | 380.6 |
|                | HLA-DRB1*01:01 | 577.8 |
|                | HLA-DRB1*01:01 | 198.6 |
| Sequence | Position | HLA-DRB1 Alleles | Frequency |
|----------|----------|------------------|-----------|
| NGSYYITARDM 979 YMPR | 993 | HLA-DQA1*01:02/DQB1*06:02 | 393.3 |
| GSYYITARDMYM 980 MPRA | 994 | HLA-DQA1*01:02/DQB1*06:02 | 218 |
| SYYITARDMYM 981 PRAI | 995 | HLA-DRB1*01:01 | 23.1 |
| YYITARDMYMP 982 RAIT | 996 | HLA-DRB1*01:01 | 40.8 |
| YITARDMYMPR 983 AITA | 997 | HLA-DRB1*01:01 | 145.4 |
| YYITARDMY 0.8845 | IQVNGSYYITA 976 990 |
|-------------------|---------------------|
| HLA-DRB1*08:02   | 955                 |
| HLA-DRB5*01:01   | 206.9               |
| HLA-DQA1*05:01/DQB1*02:01 | 491.6 |
| HLA-DRB1*04:01   | 723.4               |
| HLA-DRB1*04:04   | 819.7               |
| HLA-DRB1*11:01   | 72                  |
| HLA-DRB1*11:01   | 72                  |

| QVNGSYYITAR 977 991 |
|---------------------|
| HLA-DPA1*01/DPB1*04:01 | 710.8 |
| HLA-DPA1*01:03/DPB1*02:01 | 875.8 |
| HLA-DQA1*05:01/DQB1*02:01 | 292.7 |
| HLA-DRB1*03:01 | 588 |
| HLA-DRB1*11:01 | 32.4 |
| HLA-DRB1*11:01 | 32.4 |
| HLA-DPA1*01/DPB1*04:01 | 557.6 |
| HLA-DPA1*01:03/DPB1*02:01 | 860.8 |
| HLA-DQA1*05:01/DQB1*02:01 | 311.9 |
| HLA-DRB1*11:01 | 17.9 |
| HLA-DRB1*11:01 | 17.9 |

| NGSYYITARDM 979 993 |
|----------------------|
| HLA-DPA1*01/DPB1*04:01 | 503 |
| HLA-DPA1*01:03/DPB1*02:01 | 763.2 |
| HLA-DQA1*05:01/DQB1*02:01 | 387.6 |
| HLA-DRB1*09:01 | 858.7 |
| HLA-DRB1*09:01 | 858.7 |
| HLA-DRB1*11:01 | 11 |
| HLA-DRB1*11:01 | 11 |

| GSYYITARDMY 980 994 |
|----------------------|
| HLA-DPA1*01/DPB1*04:01 | 504.5 |
| HLA-DPA1*01:03/DPB1*02:01 | 790.4 |
| HLA-DQA1*05:01/DQB1*02:01 | 482.9 |
| HLA-DRB1*11:01 | 15.2 |
| HLA-DRB1*11:01 | 15.2 |
3.8 Molecular Docking

Molecular docking was achieved using peptide-binding groove affinity by docking MHCI alleles with chicken BF alleles (BF2*2101 & BF2*0401). The chicken alleles were used a receptors, and the top MHCI epitopes \(982YITARDMYM_{990}\), \(983YITARDMYM_{991}\) and \(985TARDMYMP_{993}\) were used as ligands.

Docking of \(983YITARDMYM_{991}\) epitope with BF2*2101 and BF2*0401 alleles showed – 72.11 and – 37.39 global energy respectively which indicates the strong binding affinity between the ligands and the receptors compared to other epitopes (Figs. 5, 6 and 7). In general, the global binding affinity of ligands with the receptor BF2*2101 alleles was found to be lower compared to BF2*0401, which indicates strong interaction between the receptor and ligands.

Discussion:

Acquired immunity results in the activation of antigen-specific effector mechanisms including B-cells (humoral), T-cells (cellular), macrophages, and the production of memory cells [4]. The use of B cells and T cells epitopes as a means to induce cellular and humoral immunity is likely to lead to broad based vaccines that could reduce the challenges in using of conventional attenuated vaccine [40]. There are several potential advantages offered by peptide vaccine over traditional organism vaccines. Most importantly, it allows the immune response to focus only on relevant epitopes and avoid those that lead to non-protective responses, immune evasion, or unwanted side effects, such as
autoimmunity [41].

Vaccination studies with IBV have always focused on humoral immune responses in relation to protection [4]. Acquired immunity results in the activation of antigen-specific effector mechanisms including B-cells (humoral), T-cells (cellular) and macrophages, and the production of memory cells [4]. Chickens develop a good humoral response to IBV infections, which measured by ELISA, virus neutralizing (VN) and haemagglutination-inhibition HI antibodies tests [42]. It is known that S1 glycoprotein of IBV responsible of virus neutralizing (VN) and haemagglutination-inhibition HI antibodies and has been considered as the most likely inducer of protection [4].

Recently multi peptide vaccines using immunoinformatics tools was performed in Sudan for several viral diseases in chicken such as ILTV, fowlpox, Newcastle and marek's disease virus [43-46].

In this study, the physiochemical properties of spike S protein were computed using protparam. The protein reflects good stability and antigenic nature. The secondary structure prediction by GOR IV server revealed that the protein contained alpha helix (29.43%), extended strand (27.37%), beta turn (5.25%), and random coil (37.95%). DiANNA1.1 tool calculated 19 disulphides bonds (S-S) positions and the trans-membrane protein topology using TMHMM tool revealed that residue from 1 to 1093 were exposed on the surface, while residue from 1094 to 1116 were inside trans-membrane-region and residues from 1117 to 1162 were buried within the core-region of the S protein. In addition, Corona-S1 and Corona S2 domains were identified in refseq of IBV spike glycoprotein using Conserved Domain (CDD) BLAST search. The top associated sequences in both domains were Feline infectious peritonitis virus (strain 79-1146), Avian infectious bronchitis virus (strain Beaudette), and Human coronavirus 229E whereas Sever acute respiratory syndrome- related coronavirus sequences was only related to corona-S2 domain.

In B cell methods, seven shortened conserved epitopes (1139KKSSYY1144, 1140KSSYYT1145, 1141SSYYT1146, 1141SSYYT1145, 1142SYYTT1146, 1142SYYTT1145, and 1143YYTT1146) were predicted from B cell prediction methods as surface, linear and antigenic epitopes. These epitopes were adjacent to each other from the position 1139-1146. This result is consistent with the results of conventional
vaccines studies. [42]. In a similar study, using BepiPred epitope prediction server version 1, only one epitope (YTSNETTDVTS\textsuperscript{175-185}) was predicted within the S1 glycoprotein of M41 IBV strains and three such epitopes (VSNASPNSSGVD\textsuperscript{279-290}, HPKCNFRPEN\textsuperscript{328-338}, NETNNAGSVSDCTAGT\textsuperscript{54-69}) were predicted in CR88 IBV strains [40]. Linear B cell epitopes have been reported to play a role in virus neutralization [40]. IEDB prediction tool was used to predict linear, surface and antigenic epitopes based on the properties of amino acids such as hydrophilicity, surface accessibility, flexibility, and antigenicity [43].

Cytotoxic T lymphocytes (CTL) provide a critical arm of the immune system in eliminating autologous cells expressing foreign antigen. Unlike humoral immunity, the specificity of CTL activation depends on membrane receptors rather than secreted molecules, and antigen receptors of CTL interact with peptide determinants only in association with matched major histocompatibility complex (MHC) molecules. Virus-specific CTL have been shown to be important, if not critical, for resolution of infection and elimination of viral shedding [1]. It is stated that, the major histocompatibility complex MHC restricted CTL response can be associated with decreases in viral load, CD8\textsuperscript{+} lymphocytes were mostly responsible for the observed protection [1, 47]. Cytotoxic T-lymphocyte (CTL) responses to infectious bronchitis virus (IBV) were determined at regular intervals between 3 and 30 days post infection [1].

However, MHCI prediction methods showed three conserved CTL epitopes \textsubscript{985}TARDMYMPR\textsubscript{993}, \textsubscript{983}YITARDMYM\textsubscript{991} and \textsubscript{982}YYITARDMY\textsubscript{990} as they linkage with 7 and 3 human MHCI alleles respectively and showed high antigenicity, no allergenicity and no toxicity. Recent studies showed that vigorous cytotoxic T lymphocyte (CTL) responses that correlate with initial decrease in infection and illness can be detected after IBV infection [47]. It was established that the CD8\textsuperscript{+} T cells were exhausted without CD4\textsuperscript{+} helper T cells. CD4\textsuperscript{+} T cells do not appear to be important in initially resolving IBV infection in chickens [47].

In MHCI prediction method, several core peptides were predicted to interact with MHCI alleles, but surprisingly the top core peptides were also \textsubscript{983}YITARDMYM\textsubscript{991} and \textsubscript{982}YYITARDMY\textsubscript{990} which were
presented in MHCI prediction methods. They linked with 52 and 38 human alleles respectively. These epitopes were showed high antigenicity, no allergenicity and no toxicity. Moreover, the physiochemical properties of spike protein were also analyzed confirmed that protein has appositively charge and stable.

Molecular docking was performed to display the interaction between BF alleles (BF2*2101 & BF2*0401) and predicting MHCI epitopes (\textsubscript{982}\textit{YYITARDMY}_990, \textsubscript{983}\textit{YITARDMYM}_991 and \textsubscript{985}\textit{TARDMYMPR}_993). The 3D structures of MHC class I binding peptides were designed using PEPFOLD and docked with BF alleles via Patchdock server. Docking of \textsubscript{983}\textit{YITARDMYM}_991 epitope with both BF2 alleles produced strong binding affinity (−72.11 and −37.97 global energy respectively) followed by \textsubscript{982}\textit{YYITARDMY}_990 (−64.68 and −37.57 global energy respectively). This indicates the strong interaction between the ligand and the receptor compared to other epitopes (see Figs. 5, 6 and 7). The interaction of ligands with the receptor BF2*2101 alleles was stronger compared to BF2*0401. The predicted epitopes should be tested for therapeutic potency in future studies to prove their safety and efficacy.

Conclusion:
Peptide vaccine was found to be an effective and powerful approach to a variety of pathogens. Peptides may have the potential to act as safe, non-infective, well-specific, stable vaccines. Peptide-based vaccine can correspond to highly conserved regions required for the pathogen's function and can elicit both humoral and cellular immune responses.

In this study, five epitopes were predicted from spike glycoprotein of IBV as the best B cell (\textsubscript{1139}\textit{KKSSYY}_1144, \textsubscript{1140}\textit{KSSYYT}_1145 and \textsubscript{1141}\textit{SSYYT}_1145) and T cell epitopes (\textsubscript{982}\textit{YYITARDMY}_990 and \textsubscript{983}\textit{YITARDMYM}_991) due to high antigenicity, no allergenicity and no toxicity as well as great linkage of MHC epitopes with MHCI MHCI alleles. These epitopes should be designed and incorporated and then tested as multi-epitope vaccine against IBV and may act as a potential peptide vaccine to control IBV infection in chicken by inducing humoral and cellular responses.

Peptide vaccine against IBV spike protein (S) is strongly supersedes the conventional vaccines, as it
designed to cover all strains in different serotypes, which might be reduce the frequent outbreaks and their huge accompanied economical loss to a minimum.

**Abbreviations**

IB: Infectious bronchitis; IBV: Infectious Bronchitis Virus; IEDB: Immune Epitope Database; S: spike; MHC: major histocompatibility complex; BF: The genetic polymorphism of properdin factor B; refseq: reference sequence; NCBI: National Central Biotechnology Information; MSA: multiple sequence alignment; GRAVY: Grand average of hydropathicity; CDD: Conserved Domain Database; IC50: The half maximal inhibitory concentration; ANN: artificial neural networks; NN-align: artificial neural network-based alignment; HLA: The human leukocyte antigen; CTL: Cytotoxic T lymphocytes.

**Declarations**

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**Authors’ contributions**

Eman, A. Awadelkareem and Sumaia A. Ali designed this study, accomplished the experiments and analyze the results. Sumaia A. Ali interpreted the data and wrote the manuscript. All authors read and approved the final manuscript.

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Figures
Figure 1

(a): The secondary structure of IBV spike protein; (b) transmembrane topology of spike protein; (c) the position of disulphides bond (S–S) in spike protein of IBV.

Figure 2

Multiple sequence alignment of spike (S) protein of IBV visualized by Jalview 2.10.5. Yellow color bar and star sign indicate the full conservation. The brown region indicates the mismatched sequences among them. Black bars show the consensus logo sequence and yellow color indicates good quality.
Figure 3

Phylogenetic tree of retrieved strains of Spike protein using MEGA7.0.26 software.
Figure 4

(a): The 3D structure of Spike (S) glycoprotein of IBV using chimera picturing tool. (b) The position of proposed MHCI and MHCII epitopes of IBV (green colour) illustrated by UCSF-Chimera visualization tool.

| Peptide   | Receptor | Energy | Attractive vdw |
|-----------|----------|--------|----------------|
| YITARDMYM | BF₂ 2101 | -72.11 | -37.79         |
|           | BF₂ 0401 | -30.33 | -38.52         |

Figure 5

Docking of YITARDMYM with BF2 alleles
| Peptide     | Receptor | Energy | Attractive vdw |
|-------------|----------|--------|----------------|
| YYITARDMY   | BF₂ 2101 | -64.68 | -35.26         |
|             | BF₂ 0401 | -37.57 | -23.93         |

Figure 6
Docking of YYITARDMY with BF2 alleles

| Peptide     | Receptor | Energy | Attractive vdw |
|-------------|----------|--------|----------------|
| TARDMYMPR   | BF₂ 2101 | -45.61 | -30.25         |
|             | BF₂ 0401 | -35.47 | -28.81         |

Figure 7
Docking of TARDMYMPR with BF2 alleles