IN-SILICO MULTIEPITOPE VACCINE DESIGN AGAINST H7N9 INFLUENZA VIRUS

Project report submitted in partial fulfilment of the requirements for the award of the degree of

MSc in BIOINFORMATICS

By

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DECLARATION BY THE STUDENT

I, Miss. MANJUABHINAYA VR. hereby declare that the project report, entitled "IN-SILICO MULTIEPITOPE VACCINE DESIGN AGAINST H7N9 INFLUENZA VIRUS" submitted to Garden City University, in partial fulfilment of the requirements for the award of the Degree of Master of Science in Bioinformatics is a record of original and independent research work done by me during the year 2023, under the guidance and supervision of Dr.Shanmugha Priya , Department of Life Sciences, and it has not formed the basis for the award of any Degree Diploma/Certificate or any similar title to any candidate in any University.

Signature of the Candidate

Place: Bangalore, Karnataka

Date: 22nd August 2023

Register Number: 22MSBI103

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Thank you

Date: 22nd May 2020 Signature of the candidate

CERTIFICATE

This is to certify that the project work entitled "IN-SILICO VACCINE DESIGN OF A MULTIEPITOPE VACCINE AGAINST H7N9 INFLUENZA VIRUS" is a bonafide work of Mr./Ms. MANJUABHINAYA VR bearing University Register Number 22MSBI103 and is being submitted in partial fulfilment for the award of the Degree Master of Science in Bioinformatics at Garden City University.

Date:22nd August 2023

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ABSTRACT

Avian influenza A(H7N9) is a subtype of influenza viruses that have been detected in birds. Despite recent advances in the diagnosis and treatment for H7N9 , it is one of the devastating viral infection with high mortality rate. oseltamivir (Tamiflu) or zanamivir (Relenza) have been used off-label to treat severe cases of H7N9 influenza is the current drug available for its treatment. With the recent advances made in the field of immunoinformatic tools, combined with the knowledge of host immune responses has led to new disciplines in vaccine design against diseases via in silico epitope predictions. The identification of specific epitopes derived from various H7N9 strains/species would significantly enhance the advancement in the development of peptide-based vaccines. Using computational tools, in this project we have screened 2 strains and identified the most immune dominant HLA class I, HLA class II and B-cell epitope. By further analyses, 14 promising epitopes were selected, which were later joined together using molecular linkers to create a multivalent recombinant protein against influenza virus To increase its immunogenicity, we added a 50 S ribosomal protein L7/L12 (Locus RL7_MYCTU) as an adjuvant to its design. A major advantage of this current vaccine approach is it's multivalent nature (recognizing multiple-epitopes), which is likely to provide enhanced protection against complex influenza agents . Here, we describe the computational analyses leading to in silico multi-epitope peptide vaccine design.

Key words: Hemagglutinin, Neuraminidase , multi -epitope peptide vaccine

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IAV/AIV - Influenza Avian Viruses

HA- Hemagglutinin

NA- Neuraminidase

B cell- BepipredLinear cell prediction

T Cell- Toxic

MHC- Major histocompatibility complex

1. INTRODUCTION

The Avian influenza A (H7N9) which is a subtype of influenza A virus (IAV) that has been detected in birds it is also referred as bird flu virus. H7N9 virus usually circulate among Avian populations with some variants known to occasionally infect humans .H7N9 was first reported to have infected human in China on March 2013 [1][2]. Influenza A virus is a negative single stranded RNA virus that belongs to the family Orthomyxoviridae [3]. It is said that influenza virus tends to strike during the winter than summer.

The World Health Organization (WHO) has identified H7N9 as H7N9 is one of the dangerous viruses for humans [3]. Since then, H7N9 infections has continued occur during five waves of epidemics. The virus has caused increased mortality in any epidemic periodic by causing acute respiratory distress syndrome, especially in the elderly people (.60 years of age) [4][5].

H7N9 causes severe disease symptoms including fever, cough and shortness of breath which may lead to severe pneumonia, in few cases blood poisoning and organ failure are also possible. And the main part of this virus infection is there was sex difference exist in H7N9 was males are more susceptible to infection than females [6]

Human infection with any H7 influenza virus is rare and H7N7, H7N3 and H7N2 are the only subtypes that were found to cause human infections previously. The emergence of a novel reassortment avian influenza virus causing fatalities among humans is a significant threat for public health because if it spreads through huma-to-human transmission it can lead to deadly pandemics.[7]

There are eight segments in H7N9 are HA, NA, NEP (nuclear proteins), Matrix proteins (M2), Nucleocapsid protein (9NP), Polymerase PA (PA), Polymerase PB1 (PB1) and Polymerase PB2 (PB2).H7N9 has majorly two surface glycoproteins or envelope proteins are Hemagglutinin (HA), of which there are 18 subtypes (H1-H18) Neuraminidase (NA), of which there are 11 subtypes (N1-N11) [8][10].

Hemagglutinin is class I fusion protein, having multifunctional activity as both an attachment factors a membrane fusion protein. Therefore, HA is responsible for binding Influenza virus to sialic acid on the surface of target cells, such s cells in the upper respiratory tract or erythrocytes causing as a result the internalization of the virus. HA proteins(hemagglutinin) is a major glycoprotein which plays crucial role in the early stage of virus infection and it is also responsible the binding of the virus to cell surface receptors and it mediates liberation of the viral genome into the cytoplasm through membrane fusion [9].

A major function of Neuraminidase NA occurs in the final stage of infection. Viral NA removes sialic acids from both cellular receptors and from newly synthesized HA and NA on nascent virions, which have been sialayted as part of the glycosylation processes within the host cell. NA cleavage of sialic acids prevents virion aggregation and stops virus binding back to the dying host cell via the HA, enabling efficient release of virion progeny and spread to new cell target [10].

Several vaccines have been developed till now for the prophylaxis against the Human IAV with the main target of HA. But however, the function these vaccines are limited due to the high mutation rate in the antigenicity of HA. Sometimes the antigenicity of the vaccines does not match for the epidemic viruses Prediction accuracy has decreased because of random genetic drift, incomplete samples of viruses that cause epidemics, and lack of knowledge regarding the evolution mechanism of sequences.

From last decade the complex calculations has been developed for predicting virus lineages detecting genetic variations and their functional impact so this technique such as in-silico trials has been instrumental for vaccine design

In this work, we evaluated the conserved parts of HA, NA among the seven pathogenic strains, especially in Asia: H1N1, H1N2, H3N2, H7N9 and H9N2 by in silico method and combination as a single protein that can activate human humoral and cellular immunity silico method and combination as a single protein that can activate human humoral and cellular immunity [10].

So, the in-silico technique modelling is a useful approach for reducing the cost and time required for the drug development. In the current study, we used docking studies to perform in silico-based vaccine against influenza A (H7N9).

2. Literature Review

The Historic origin of vaccine designing has been traced back in 1796 the first vaccine was developed during the period of 1930-1950 by Edward Jenner against the small pox. Till now there are several vaccines has been constructed against many viruses including H7N9. By many advance studies in molecular Biology and Immunology which led to the modern era of subunit vaccine development.

The first licensed subunit was designed in the year of 1986 was recombinant hepatitis B vaccine and further in 19^{th} and 20^{th} century the advances in vaccine design was carried out by researchers such as Pasteur, Koch, von Behring, Calmette etc.

The traditional dogma of immunology is that all vertebrate has both innate and adaptive immunology. The innate immune system acts more rapidly, less specificity and works as the first line defence such as skin, sweat etc whereas adaptive immune system comprises of cellular immune response of T cells and humoral response of B cells. B cell epitopes can be linear and discontinuous amino acids while the T cell epitopes are short linear peptides. Most of the T cells can be either CD8+ or CD4+that is distinguished by the presence of one or the other of the two glycoproteins on their surface.CD4T cells function as T helper cells (TH) cells that recognize peptide s displayed by MHC class II molecules.

From the year 2013 the outbreak of influenza virus H7N9 has been occurred in China and most recent in the year of 2021 it has been occurred in China itself H7N9 avian influenza continued to cause sporadic outbreaks in subsequent years, with cases reported in different provinces.

It is transmitted from birds to humans. Th e virus mainly circulates among birds, particularly poultry without causing significant illness in them. Human infections occur through direct or indirect contact with infected birds or contaminated environments. There is limited evidence to human-to-human transmission and most causes from exposure to live poultry markets, where the virus can to present on surfaces, in the air and in the feathers or excretions of infected birds. Preventions are surveillance and early detection, control of poultry markets and farms, Antiviral treatment etc.

The advances made in the field of immunoinformatic tools, combined with the knowledge about host immune responses via in silico epitope predictions has led to new disciplines in vaccine design against diseases. The identification of specific epitopes derived from various H7N9 strains would significantly enhance the advancement in the development of peptide-based vaccines. Thus, we intent to extend the analysis to design a safe multi-epitope vaccine for H7N9 strainsthat causes Influenza viruses in humans

3. Methodology

3.1 Retrieving influenza protein sequences and structure analysis

The protein sequence was retrieved from National Center for Biotechnology Information (NCBI) in Genbank was used for the selection and the retrieval of full FASTA file of Influenza A (H7N9) strains of two surface glycoproteins: Hemagglutinin and Neuraminidase of the influenza A (H7N9) strain were chosen. NCBI protein BLAST (https://www.ncbi.nlm.nih.gov/) was used to look for homologous sequence in Influenza A virus (A/Shanghai/4664T/2013(H7N9)) and (A/Shanghai/4664T/2013(H7N9))] as these stereotypes are the most commonly associated with the human infection case.

3.2 Multiple Sequence Alignment (Conserve regions analysis)

Clustal omega server (https://www.ebi.ac.uk/Tools/msa/clustalo/) was then applied to specify the conserved regions among the homologous sequences for Hemagglutinin protein (NCBI protein ID: UXD21033.1 , AGI60292.1 , AIZ70019.1 , YP_009118475.1 , AGI60301.1) and for Neuraminidase protein (NCBI protein : AGI60295.1 , AHH30761.1 , AHH30773.1 , YP_009118481.1 , AGI60300.1) followed by that the Analysis of transmembrane regions for both proteins HA and NA has been performed using DEEPTMHMM tool Likely the TMHMM server, based on a hidden Markov model can correctly predict of transmembrane protein topology that makes it unique from other methods(**Fig:1**)

3.3 Anticipating and Screening of B cell of linear epitopes

B cell epitope prediction is performed in IEDB Each predicted epitope is given a score known as protrusion index value by the server. For each protein, a number of epitopes has been predicted. The main objective of b cell epitope prediction is to identify a potential antigen that will interact with B cell epitope prediction is to identify a potential antigen that will interact with b lymphocytes and trigger an immunological response. The antigenicity of the peptide is connected to its flexibility, according to the experimental study. Being a possible cell epitope also necessitates proper surface accessibility. Thus, b cell epitope prediction tools from IEDB with accuracy of 75% was used to identify the B cell(**fig2**)

3.4 Assessment of T cell epitopes and analysis of Antigencity, allergicity and toxicity of peptides

MHC class 1 (HLA A and B and MHC class 2 (HLA DR, DP and DQ) alleles are applied to generate cytotoxic T – cell (CTL) and helper T cell (HTL) epitopes, respectively using the IEDB MHC prediction tool was used to assess the analysis of epitopes binding to MHC -1 molecules. This server applied artificial neural network, stabilized matrix method or scoring matrices derived from combinational peptide libraries to generate the prediction method.

Following that the epitopes antigenic scores and topology were checked using Vaxijen v2.0 server. Vaxijen v2.0 server transforms protein sequences using auto cross covariance into unform vectors of principal amino acid properties and can predict accurate result.

Based on the percentile score, higher vaxijen score and topology the best epitopes were elected. epitope was consideredAndallergicity from allertop2.0 and the toxinpred2.0 server (https://webs.iiitd.edu.in/raghava/toxinpred2/) was used to affirm the toxicity of the selected epitopes and the interferon-gamma inducing epitopes. IFNepitope is a webserver that allows users to identify IFN-gamma inducing MHC class I and II binding in a peptide/antigen (http://crdd.osdd.net/raghava/ifnepitope/) **(Table:1, Table: 2)**

3.5 Construction of Vaccine

T cell epitopes other epitopes were used to construct vaccines . The Vaccine were created by combining different adjuvants with sequences and the best HTL epitopes, CTL epitopes and BCL epitopes . TheEAAAK, AAY, GPGPG, and HHHHHH linker were used to connect all of the epitopes and adjuvants because it keeps the domains equal.(**Fig:3**)

3.6 Population coverage and conservancy analysis

HLA genes are distributed differently among ethnic groups and geographical regions around the world population coverage must be considered among while developing a potential vaccination to cover the largest possible population keeping all the parameters default. To investigate the coverage population for each epitope, the IEDB server's population coverage analysis tool (http://tools.iedb.org/population/) had been used. This tool is used to calculate the percentage of individuals estimated to respond to a given epitope set based on HLA genotypic frequencies and MHC binding or T cell restriction data. (**fig.4**)

3.7 Analysis of physiochemical characteristics, secondary and Tertiary structure prediction, refinement and validation of constructed vaccine

The ProtParam server (http://expasy.org/cgi-bin/protpraram) was used to functionally characterize the vaccine. The physiochemical variables measured included molecular weight, aliphatic index, isoelectric pH, hydropathicity, estimated half-life, instability index and GRAVY values(**Table:3**).The SOPMA (https://npsa-pbil.ibcp.fr/cgi bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html)server has also been used to predict the vaccine proteins beta sheet, coil structure and alpha helix **(Fig5)**. is a highly accurate secondary prediction method that incorporates two feed forward neural networks that analyse PSI-BLAST output and use a stringent cross validation procedure to evaluate performance.By using alpha fold the protein structure has been generated **(Fig:6)** Moreover, Ramachandran plot analysis was performed to identify the potential errors in selected vaccine where 94.6% of favoured regions are their in the total of 514 residues .To increase the accuracy of the predicted 3D modelled structure, refinement was performed using Galaxy web (http://galaxy.seoklab.org/cgi-bin/submit.cgi?type=REFINE). And the tertiary structure validation is performed using PROSA**(Fig:7)**

3.8 molecular docking of the selected vaccine candidate

Molecular docking is a computational approach that predicts the preferred orientation of the ligand to the receptor when they are joined together to from a stable complex. A recent study has reported that docking success rate with each energy parameter set results in 100 ranked models. Here we have used HADDOCK (https://wenmr.science.uu.nl/haddock2.4/) to perform molecular docking. **(Fig:8) and the haddock score has been obtained from the Haddock server (table:4)**

3.9 In-silico Cloning and vaccine optimization

The cloning and expression efficiency of the multi-epitope peptide based vaccine construct is essential for vaccine design Because human and E. coli codons are different, JCAT, a codon adaptation tool, was utilized to apply the codon to E. coli in order to boost expression. The series lacked the BamHI and Hind III restriction sites, making cloning more appealing. The adopted sequence was then inserted between the BamHI and HIND III restriction sites in the Pet28a(+) vector. The final clone was 6900 bp long . (**Fig:9**) SnapGene has been used for cloning

4. Results

4.1. Retrieval of protein sequences and conserve regions analysis

The full protein sequence of AIV was extracted from NCBI Database (HA: AGI60292.1, NA: AGI60295.1) and performed Blast to check the homologous strains of these two surface HA and NA glycoproteins and performed clustal omega for the conserved region analysis were further study after examining the antigenic score with the overall prediction values of 0.5576, 0.7377 respectively due to their higher immunogenic potentials

Fig:1 MSA conserved regions of Hemagglutinin and Neuraminidase

4.2 Prediction and assessment of linear B-lymphocyte epitopes.

The sequence of surface protein was analysed using bepipred linear epitope prediction to identify the highest antigenic proteins. The b-ell epitope prediction, method is the best as it shows significant results both on epitope data derived from a large collection of linear epitopes from IEDB database (**Fig:2**) the few epitopes were immunogenic but toxic

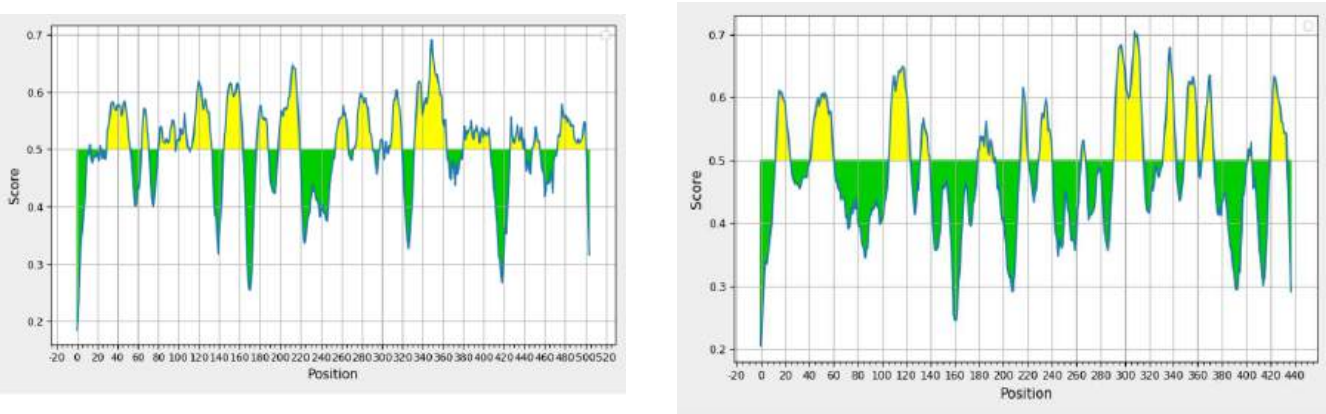


Fig:2 Graphical Representation of B Cell Epitopes Hemagglutinin And Neuraminidase

4.3 Anticipating of T cell epitopes and testing of epitopes allergenicity, antigencity and toxicity

A large number of immunogenic epitopes were identified as potential T cell epitopes for any protein that can adhere to a significant percentage of HLA-A and HLA-b Alleles using IEDB's MHC class I and II binding predictions. Epitopes with a high binding sensitivity and the ability to interact with as large variety of HLA were chosen. The epitopes were shown to be non-toxic and non-allergenic and antigen**(Tables 1 and 2**).

PEPTIDES	TOXICITY	GAMMA PRED	NON ALLERGEN	ANTIGEN	SCORE
LVAMENQHTI	NON-TOXIC	POSITIVE	NON-ALLERGEN	ANTIGEN	0.9066
MASIRNNTYD	NON-TOXIC	POSITIVE	NON-ALLERGEN	ANTIGEN	0.8701
ETNITNIQM	NON-TOXIC	POSITIVE	NON-ALLERGEN	ANTIGEN	1.3158
ETNITNIQME	NON-TOXIC	POSITIVE	NON-ALLERGEN	ANTIGEN	1.3947
NITNIQMEE	NON-TOXIC	POSITIVE	NON-ALLERGEN	ANTIGEN	1.4857
TNITNIQMEE	NON-TOXIC	POSITIVE	NON-ALLERGEN	ANTIGEN	1.3144
TNITNIQME	NON-TOXIC	POSITIVE	NON-ALLERGEN	ANTIGEN	1.524

Table:1 MHC1 epitopes Of Hemagglutinin and Neuraminidase PROTEIN (T-CELL PRED)

PEPTIDES	TOXICITY	GAMMA PRED	NON ALLERGEN	ANTIGEN	SCORE
MTKSYKNTRKSPALI	NON-TOXIC	POSITIVE	NON-ALLERGEN	ANTIGEN	0.8512
ENQHTIDLADSEMDK	NON-TOXIC	POSITIVE	NON-ALLERGEN	ANTIGEN	0.6999
QMTKSYKNTRKSPAL	NON-TOXIC	POSITIVE	NON-ALLERGEN	ANTIGEN	0.7389
PQMTKSYKNTRKSPA	NON-TOXIC	POSITIVE	NON-ALLERGEN	ANTIGEN	0.5496
SYNAELLVAMENQHT	NON-TOXIC	POSITIVE	NON-ALLERGEN	ANTIGEN	0.76
VTVGSSNYQQSFVPS	NON-TOXIC	POSITIVE	NON-ALLERGEN	ANTIGEN	1.0415
NRPVIQIDPVAMTHT	NON-TOXIC	POSITIVE	NON-ALLERGEN	ANTIGEN	0.7918
NNYYNETNITNIQME	NON-TOXIC	POSITIVE	NON-ALLERGEN	ANTIGEN	1.0803
TIHDRSQYRALISWP	NON-TOXIC	POSITIVE	NON-ALLERGEN	ANTIGEN	0.6388

Table 2: MHC-2 epitopes OF Hemagglutinin and Neuraminidase PROTEIN (T-CELL PRED)

4.4 Vaccine construct by mutiepitopes

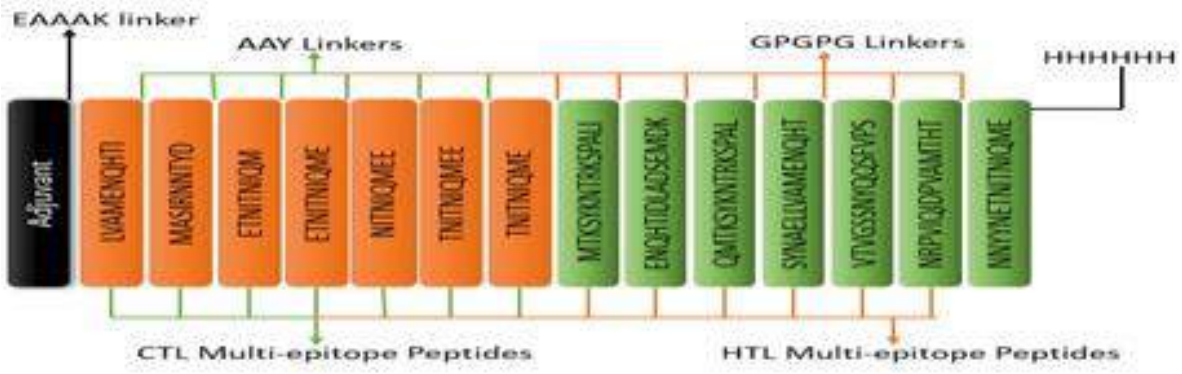


Fig:3 epitopes with high score non allergen nontoxic and antigen attached with adjuvants and linkers

4.5Prediction of vaccine physiochemical parameters

Property	
Number of amino acids	514
Molecular weight	55879.14
Theoretical pi	9.62
Total number negatively charged	45
Total number of positively charged	64
Extinction co efficient (assuming all pairs if cys residues from cysteines	40800
Instability index	38.67
Aliphatic index	68.58
Grand average of hydropathicity	-0.690

Table:3 Physiochemical properties

The ProtParam tool from the Expasy service was then used to characterize the physiochemical properties of the chosen vaccination candidate. The molecular weight of the produced vaccine construct was 55879.14, indicating that it had substantial immunogenic potential. The protein would have the above net negative charge if its theoretical pI was

9.62, and vice versa. If all cysteine residues were removed, the extinction coefficient was 40800 at 0.1% absorption (**Table 3**). The protein's hydrophilic behaviour and thermostability are demonstrated by the GRAVY value (− 0.690) and aliphatic index (68.58). The protein's physicochemical characteristics indicated that it was stable and capable of eliciting a significant immune response from the body

4.6 Measuring of population coverage and MHC restricted alleles conservancy analysis

The assessment resource IEDB population covering computation tool demonstrated population coverage at each unique epitope. The demographic range of all the alleles displayed in (**Fig:4**) was computed after they were shown to be effective binders with the specified epitopes. Two different inhabitant coverages were computed for MHC class I and II restricted peptides, employing CTL and HTL populations respectively.

population/area	Class combined		
	coverage[a]	average_hit[b]	pc90[c]
World	47.76%	1.04	0.19
Average	47.76	1.04	0.19
Standard deviation	0.0	0.0	0.0

[a] projected population coverage
[b] average number of epitope hits / HLA combinations recognized by the population
[c] minimum number of epitope hits / HLA combinations recognized by 90% of the population

Population: World

MHC class	Coverage	Average hit	PC90
combined	47.76%	1.04	0.19

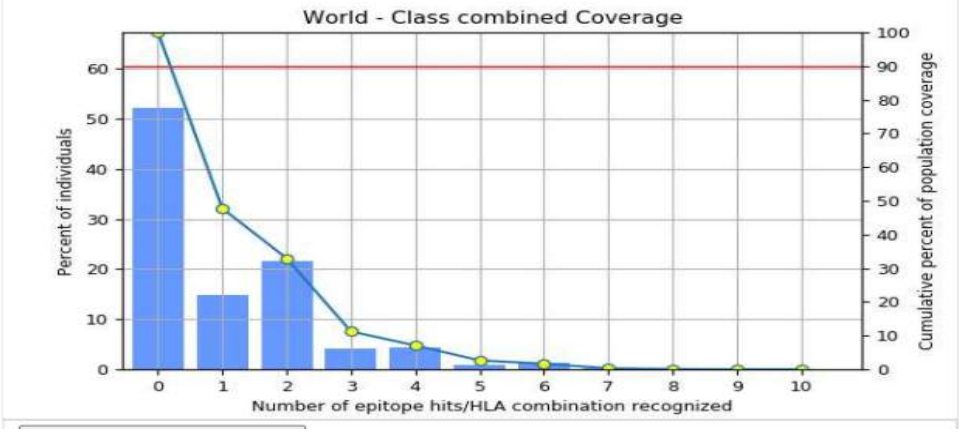


Fig: 4 Population coverage of the MHC 1 and 2 epitopes

4.7 secondary structure predictionof the multiepitope vaccine

The secondary structure of the final vaccine construct was predicted from omline server SOPMA. It predicts the secondary structure on the basis of the amino acid sequence of the proteins 514 amino acids long construct analysed in which 259 amino acid is involved in coil formation and 99 amino acids are involved in and alpha helix formation while 124 amino acids have formed beta strands. Overall secondary structure prediction results indicates(50.39%) Coils, strand are (24.12%), (19.26%) are helix (**Fig:5)**

Fig:5 Secondary structure prediction

4.8Tertiary structure prediction

The tertiary structures of the vaccine candidate generated by alpha fold were shown in**(Fig:5)**Alpha fold is used to generate the 3D structure of the multiepitope vaccine. Also it provides several level of user interaction such as template selection, alignment and model building are done completely automated by the server.

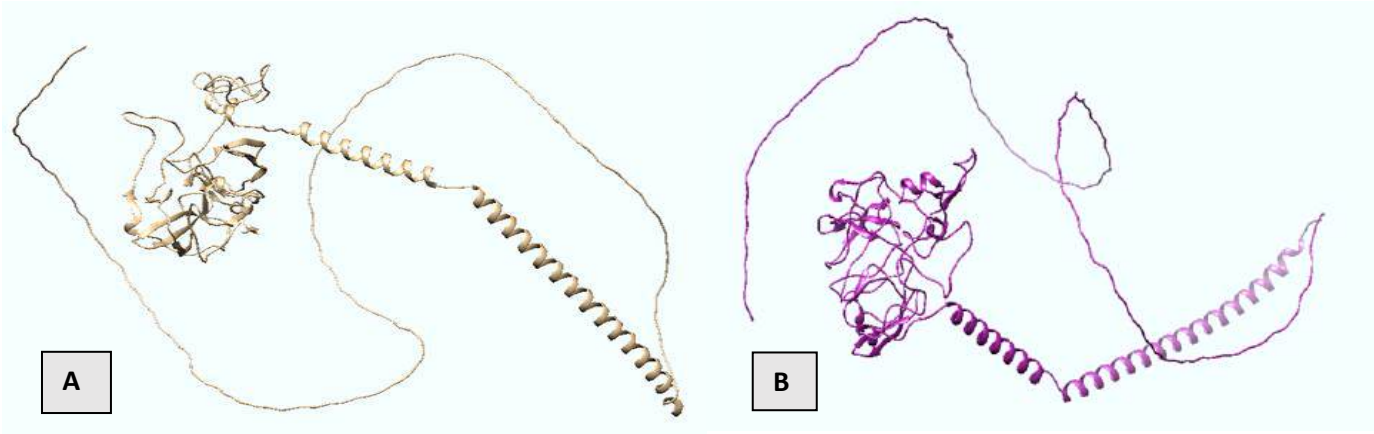


Fig 6 : 3D Protein Structure generated from (A)alpha fold and, (B) refined in galaxyrefinement

4.9 Tertiary structure validation

The Ramachandran plot analysis of the modelled protein revealed that 90.4 % of residues in the protein are in favoured regions. This is consistent with the 90.4% score predicted by the GalaxyRefine analysis. The quality and potential errors in the crude 3D model were verified by PROSA-web. The chosen model after refinement with ProSA-web gave a Z-score of −6.03 for the input vaccine protein model. The protein fell outside the score range commonly found for native proteins of comparable size(**Fig:7**)

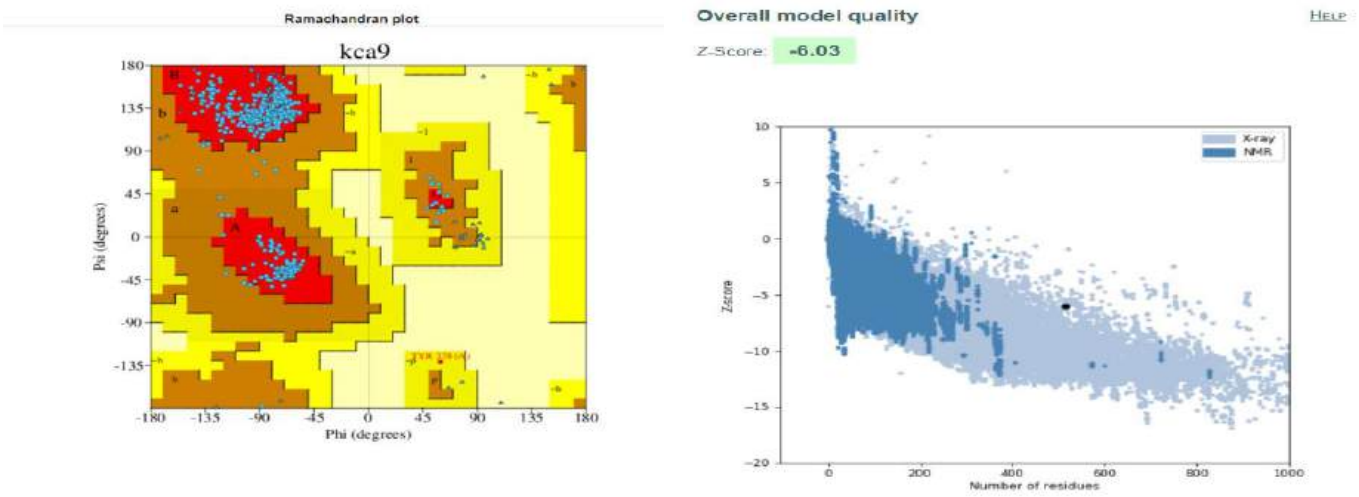
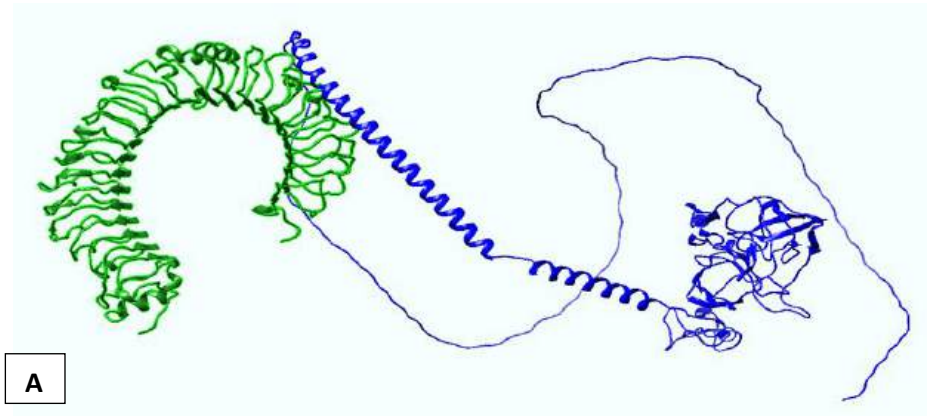


Fig: 7 Validation of the 3D structure of vaccine protein by Ramachandran plot analysis depicting 94.6% of the residues to be in most favoured regions score and Prosa web giving the z score of-6.03 the tertiary structure protein

4.10 Molecular docking

Docking against several HLA allelesresulted in successful valid modelling and model consistency evaluation having docked with TLR 4 and binds efficiently (**Fig.8**) TLR4 agonists' ability to activate dendritic cells and thus elicit Th1 and CD8+ T cell responses can be used to improve peptide-based vaccine efficacy.



Then the protein has been docked with MHC-1 and MHC-2 epitopes performed in HADDOCK (**Fig:8**)

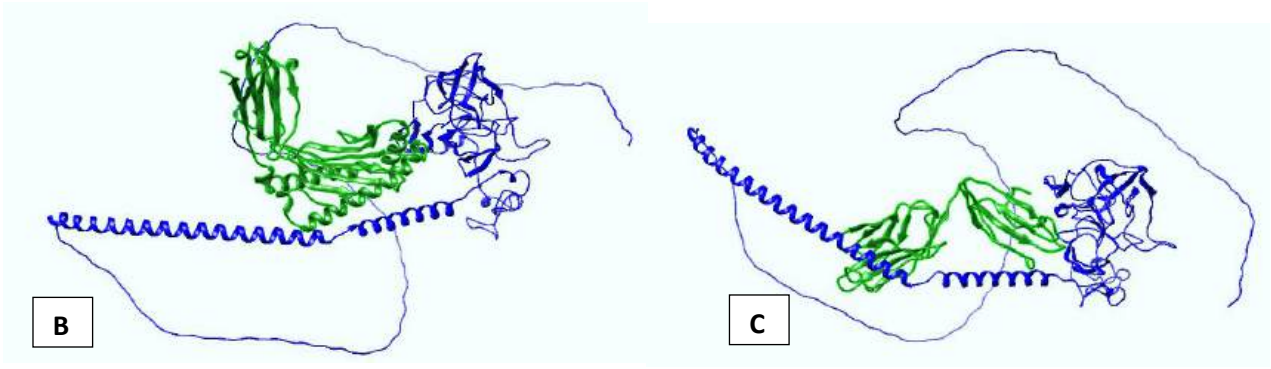


Fig:8 Docked complex of vaccine construct with humanTLR4Green colour beta sheets are the hydrogen bonds and blue one is the vaccine protein

	Molecular docking of designed chimeric protein with TLR-4	Molecular docking of designed chimeric protein with MHC-1	Molecular docking of designed chimeric protein with MHC-2
Haddock score	159.8+/- 10.0	371.2 +/- 11.9	268.5 +/- 19.9
Cluster size	6	4	17
RMSD from the overall lowest energy structure	5.2 +/- 0.2	27.1 +/- 0.1	19.3 +/- 0.2
Van der wals energy	-88.0 +/- 6.9	-57.4 +/- 7.4	-68.9 +/- 7.5
Electrostatic energy	-246.0 +/- 52.4	-234.4 +/- 54.3	-416.2 +/- 15.2
Desolvation energy	6.0 +/- 5.5	7.7 +/- 5.1	8.0 +/- 2.8
Restraint's violation energy	2910.0 +/- 139.0	4677.6 +/- 82.0	4125.5 +/- 209.8
Buried surface area	3063.3 +/- 147.4	2153.7 +/- 101.5	3011.3 +/- 90.3
Z score	-1.0	0.0	-1.3

Table:4 Haddock result retrieved from Haddock server

Here the haddock score for TLR4 is 159.8=/-10.0 ,MHC1(371.2+/-11.9), 268.5+/-19.9 with the clustal size of 6,4,17 of TLR4, MHC1 and MHC2

4.11 In-silico restriction cloning of final multiepitope vaccine construct into pet 28a, expression vector

The optimized nucleotide sequence of the final vaccine construct was cloned into the E. coli pET-28 (+) vector using SnapGene tool. HindIII and BamHI are the restriction sites were introduced to the N-terminal and the C-terminals of the sequence respectively.(**Fig:9)**

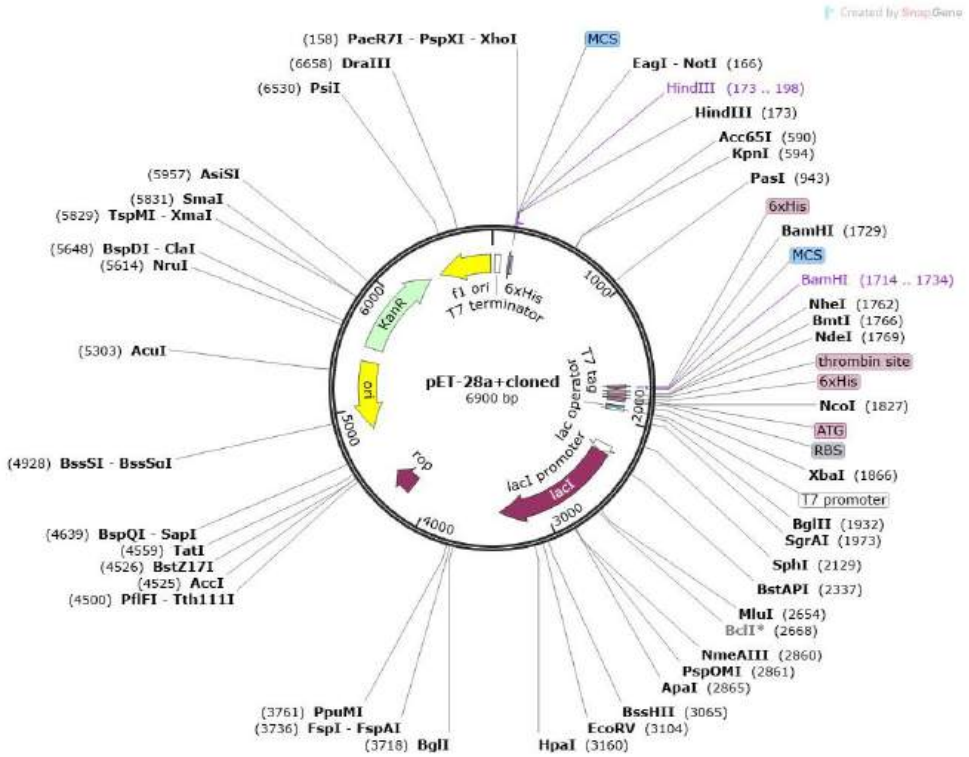


Fig:9 In-silico cloning of H7N9 nucleotide sequence insertion in Pet28a(+) by Snap Gene

5. Discussion

Multiple viral epidemics have emerged in recent years, posing an invisible thereat to both humans and animal populations. Furthermore, reassortment between different subtypes of the same virus not only aggravates the situation but also merges a new subtype, of which AIV-A (H7N9) is one. Thus, to deter the severity of deadly AIV-A (H7N9), vaccination is considered as the most fundamental and safest way. Recent advances in immunoinformatics, Bioinformatics and structural vaccinomics have re revolutionized antigen screening and aided a novel vaccine development strategy to construct vaccines against a wide range of bacterial and viral infections.

In the present study, we used the NCBI database to retrieval viral two outer proteins of AIV-A (H7N9) strains after a thorough literature analysis The ProtParam server was used to analyze the physiochemical characteristics of viral proteins. Moreover, the Vaxijen server assessed all of the retrieved protein sequences in order to find out the most potent antigenic protein and ability to confer immunity. Two viral proteins including, HA (Accession ID:AGI60292.1) and NA (Accession ID:AGI60295.1) are the surface glycoprotein responsible for binding to host cell.

Vaccines cause B cells to produce antibodies, which then mediate effector actions by engaging specifically with a pathogen or toxin. Given their involvement in retaining memory cells, longer life and guarding against infection. Most antigens and vaccinations elict a cell response in addition to a B cell. Another important immune cell type that obtains Th1 or Th2 phenotypes and drives immunological responses is the CD4+ T cell (HTL/MHC-II) Th1 response activates cytotoxic CD8 + T lymphocytes (CTL/MHC-I) natural killer cells, and

macrophages, whereas Th2 is responsible from B cell activation, isotype switching, affinity maturation, and antibody generation that kills external pathogens. Thus, T cell epitope-based vaccination is a unique process to induce strong immune response against infectious agents

In this study, we used the IEDB's MHC-I and MHC-II binding predictions, as well as topology screening and the VaxiJen score, to screen for potential CTL (MHC-I) and HTL (MHC-II) immunogenic epitopes of hemagglutinin, neuraminidase that can bind a large number of HLA-A and HLA-B alleles with high binding affinity. AllerTOP Online were used in this work to examine the allergenicity of T-cell epitopes of proteins and toxipred for checking the toxicity of the protein it's an online server.Aside from that, population coverage is a potential parameter in the reverse vaccinology technique. All anticipated T cell epitopes of HA, NA can cover populations from most geographic parts of the world, according to the findings(47.6%). MHC cluster analysis was also undertaken to establish the functional link between MHC variations, as MHC superfamilies play an important role in vaccine design and medication development. For identifying probable B- Cell epitopes, we employed four algorithms from the IEDB server's Linear B-cell epitope prediction method to predict amino acid scale-based methodologies. Though numerous B-cell epitope prediction approaches have been developed without very much success over the last few decades, experimental epitope determination has mostly focused on the identification of a linear B-cell epitope prediction method.

In this study, one vaccinationwas created using an approach in which each epitope was bonded separately using appropriate linkers. PADRE-containing vaccine designs also demonstrated greater CTL responses than vaccination without it, according to studies.

The vaccines were also tested for their non-allergic properties and immunogenic potential. In terms of antigenicity, solubility and allergenicity, Construct Vaccine was determined to be superior. Before predicting tertiary structure and refining the 3D model, the physicochemical characteristics and secondary structure of Vaccine were investigated. The produced vaccine construct Vaccine has a molecular weight of 5587.19, which is higher than the previously reported AIV vaccine candidate and it is revealed that an acceptable vaccine because proteins with a molecular weight of are considered to be more appropriate targets for vaccine development due to rapid purification. Furthermore, the aliphatic index and GRAVY value of the predicted vaccine candidate was and (− 0.690), which were slightly higher than the previous AIV vaccine candidate's aliphatic index and GRAVY value. The higher the aliphatic index value, the greater the thermostability and higher negative the GRAVY value, the more hydrophilic the vaccine, and thus capable of interacting strongly with water molecules. Moreover, the secondary structure of the chosen vaccine candidate had a higher coil structure than previously proposed vaccine candidates against AIV, and it is concluded that random coils play an important role in the high flexibility of proteins and may enhance antibody binding ability.

To reinforce our prediction, we tested the interaction of our vaccine design with several HLA molecules. In terms of free binding energy, build Vaccine was shown to be the best. Furthermore, docking analysis was utilized to investigate the binding affinity of vaccine

protein Vaccine and the human TLR8 receptor in order to assess the efficiency of the adjuvant used. Binding affinity is improved when the binding energy is low. After that, molecular dynamics simulation was used to determine the stability of the interactions. The interactions were found to be both flexible and stable, according to the research. After that, in silico cloning was done. In-silico cloning was successful, indicating that the vaccine may be produced in huge quantities in E. coli.

6. Summary

Influenza virus is a devastating viral disease with high mortality rates despite recent advances in its diagnosis and treatment. For decades, the mortality rate of H7N9has remained ≥40% despite conventional antiviral therapy and drugs used. For the current treatment of H7N9, the two mainCombination therapy of oseltamivir and peramivir. A wide consensus immunotherapeutic approaches have been developed mainly due to high drug-toxicity and emergence of drug-resistant influenza virus. In this research, we utilized immunoinformatics approaches for the development of a multi-epitope peptide vaccine against H7N9 strains that cause influenza virus. The immunogenicity analyses focused on identifying epitopes in the hyphal proteins, which helps H7N9 to adhere and invade epithelial cells, resulting in severe damage to the host cells. Development of multiepitope vaccine does not require culturing of viral strains as it required in traditional vaccine development approaches. In order to combine the humoral, innate and cell-mediated immunity, the multi-epitopes vaccine includes B cell epitopes as well as CTL and HTL epitopes. The CTL and HTL epitopes were merged together by using various appropriate linkers and an adjuvant at the N-terminal. Generated vaccine model was further studied for prediction of IFN-γ, also antigenicity, toxicity and allergenicity of the sequence was predicted. The molecular weight of the vaccine designed was found to be 49.9 kDa. The secondary structure analyses indicated that the protein consisted predominantly of 50.39% are coils, 19.26% are helix while 6.23 % are stranded. On further analysis, Ramachandran plot shows that most of the residues are found in the favoured and allowed regions (94.6%) with very few residues in the outlier region; this indicates that the quality of the overall model is satisfactory. In order to study stable interactions between ligand (multi-epitope vaccine) and receptor (TLR-4), MHC1 and MHC2 molecular docking was performed to validate interactions in the ligandreceptor complex. And the in-silico cloning has been performed by snap Gene.

5. Conclusion

Along with the continuing pandemic, the widespread transmission of the highly pathogenic AIV-A (H7N9) has raised new concerns. The situation is deteriorating as a consequence of migration-related dispersion. Thus, it will be implementing control measures without an effective medicine or vaccine. In this regard, current study describe a computational approach for multi-epitope-based peptide subunit vaccine prediction against AIV-A(H7N9).Based on a literature review, an immunoinformatic and structural vaccinology approach was used to build a multiepitope vaccine, which was confirmed using online tools to be an effective vaccination candidate against AIV-A (H7N9).

6. Scope for further enhancement

Vaccination is a principal and highly cost-effective means of controlling infectious diseases, providing direct protection against various infectious pathogens by conferring long-lasting immunological memory as well as inducing population-level herd immunity. With the recent advancement of Immunoinformatics, we get a better understanding of fundamental vaccine-induced protective immunity. In silico methods are used to identify desirable peptides and design multi-epitopes peptides vaccine. Further more, an in silico cloning can are be determined in the dry lab practices.

By using the information obtained from in silico predictions, one can easily construct a vaccine by using wet lab procedures. Prior to clinical trial, a pre- clinical development should be carried out. After the production, the vaccine should be maintained at cold temperatures within a precise range. Thus, avoiding temperature excursions or fluctuations. Vaccines indeed has to be stored between 2 and 8°C of safe zone, otherwise their quality is compromised, and their potency cannot be restored. Web-based comprehensive databases are also needed to collect various aspects of vaccine-related data, such as host gene markers predicting vaccine efficacy and critical pathways of vaccine-induced immune protection mechanisms.

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