Immunoinformatics Approach for Multi Components Vaccine Against *Human Immunodeficiency Virus*

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REVIEW ARTICLE

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Abstract: The human immunodeficiency virus (HIV) is a single-stranded RNA virus which is responsible for the cause of Acquired Immune Deficiency Syndrome (AIDS). An urgent need arises to establish antigen-based immunodiagnostic for earlier monitoring of HIV and the development of vaccines. Therefore an efficient vaccine design against HIV is necessary which is achievable with the use of the most current bioinformatics tools. In this study, immune-informatics approach was used to design a multi-component vaccine against HIV. The present study aims to choose and analyze different epitopes of Gag_Hv1h2, Rev_Hv1h3, Pol_Hv1b1, Pol_Hv1n5, Pol_Hv2ro, Pol_Hv1h2, Nef_Hv1br, Env_Hv1h2, Env_Hv1br, Vif_Hv1n5, Vif_Hv1h2, Vpr_Hv1h2, Vpu_Hv1br, Vpu_Hv1h2, Vpr_Hv1br, Tat_Hv1h2, Tat_Hv1br, Zep2_Mouse, Zep2_Human, Zep2_Rat using bioinformatics analysis and software. B-cell epitopes were predicted using IEDB and NetMHC webserver was used to the prediction of binding of peptides to MHC Class-I and Class II. Physicochemical analysis of vaccine showed that the molecular weight of candidate vaccine is <50000kDa with the half-life of 30 hours in mammalian reticulocytes, greater than 20 hours for yeast, and greater than 10 hours for E.coli. The chemicals formula of the vaccine was C1933H3139N653O615S9, and the theoretical pl of the candidate vaccine was 10.36. There are 400 amino acids in the protein structure. The aliphatic index is 58.00, the stability index is computed to be 41.72, and then it is classified as unstable proteins. The vaccine has two transmembrane helix, therefore no expression difficulties are predicted in the production of the vaccine. Ramachandran plot analysis by pro check showed that 92.5% of residues are in most favored regions 7.5% of residues are additional allowed regions and 0.0% of residues are in disallowed regions. The vaccine may activate humoral and cellular immune responses against of Human Immunodeficiency Virus. However, the vaccine was evaluated as non-allergenic, antigenic, soluble, and with an appropriate molecular weight and isoelectric point. This novel oral vaccine design could be a good vaccine candidate against HIV.

Keywords: Immunoinformatics, Ramachandra Plot, Z-score, Physicochemical, Multi-epitopes, Vaccine.

1. INTRODUCTION

The human immunodeficiency virus (HIV) may be a single-stranded RNA virus that is responsible to the explanation for acquired immune deficiency syndrome (AIDS). AIDS is defined either in terms of when CD4+ T cell counts below 200 cells per microliter or the occurrence of specific diseases in association with an HIV infection and the immunity of the physical body is weak. [1]. during a recent report suggested by the Centers for Disease Control and Prevention (CDC) show that 1,148,200 Americans aged 13 and older were living with HIV [2]. However, within the 2008-09 National AIDS Control Organization has reported that 2.39 million people accept HIV/AIDS in India [3]. While a newer investigation by the Million Death Study Collaborators within the British Medical Journal estimated that 1.4-1.6 million people suffer with AIDS [4]. There's an urgent need for controlling of AIDS by developing or producing rapid, eco-friendly, and price effective immunodiagnostic capability. Therefore, two sorts of HIV are characterized including HIV-1 and HIV-2. HIV-1 is that the virus that was initially discovered and thought of to be more virulent, which caused the bulk of HIV infections worldwide [5]. The lower infectivity of HIV-2 compared to HIV-1 implies that fewer of these exposed to HIV-2 are going to be infected per exposure, due to its relatively poor capacity for transmission; HIV-2 is essentially confined to West Africa [6]. The culture of HIV is in laboratory is difficult and time consuming process; thus, variety of immune-informatics tool are developed for prediction of T and B cells epitopes.

Vaccine design within the pre-omics era depended heavily on biochemical and immunological techniques like phage display library, overlapping peptides, ELISA, immunohistochemistry, immunofluorescence, radioimmunoassay, Western blotting, NMR spectroscopy, X-ray crystallography of antibody-antigen structure, and attenuation of untamed type pathogens by random mutation and serial passage, a process that's very

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expensive, time-consuming, with poor immunogenicity and therefore the possibility of reversion of attenuation. Now, with the help of epitope predictive software and databases, it's possible to narrow the main target sooner on a protein of interest, and thereby reduce drastically the need for laboratory experimentation. Hence, vaccine construction is formed both cheaper and faster [7]. Given the potential importance of epitope identification in producing vaccines against infectious, immune and other antigen-related diseases, peptide sequences are studied by researchers during a wide selection of biomedical fields, and as a consequence there has been an outsized expansion of databases, predictive methods and related software.

In silico methods for epitope prediction also are available but the bulk specialize in predicting protein residues that form a part of any epitope and thus don't recognize antibody especially [8]. Only a limited number of antibody-specific epitope prediction protocols are proposed so far [9]. The maintenance of HIV in tissue culture within the laboratory may be a difficult, skilled, time-consuming process; thus, several immune-informatics tools are developed to predict B- and T-cell epitopes of virulence-associated proteins. These programs have emerged on the idea of accessible and validated data that have specific algorithms [10]. HIV/AIDS may be a major public ill health that there's a pressing need for sensitive and specific diagnostic methods. In current practice pathologists perform immunoassays as a diagnostic measure that needs purified antigen which is attained only by culture of HIV or recombinant technology. so as to bypass this issue, bioinformatics is an alternate approach for prediction of epitopes without wet laboratory practice and which may help to accelerate virology research. As an example, T-cell epitopes could also be synthesized chemically to use as antigen in commercial diagnostic kits for HIV detection and also for development of peptide-based vaccines [11].

The technological advances within the fields of genomics, proteomics, human immunology, and structural biology have provided the molecular information for the invention and prediction by bioinformatics tools of novel antigens, epitopes, and style of vaccines against pathogenic bacteria, like meningococcus B [12, 13, 14]. Bioinformatics tools can predict sequences with binding affinity to MHC alleles and epitopes of T and B cells [15]. The main target on vaccine design and development has changed to the assembly of peptides composed of multiple epitopes (multi epitope vaccines), supported linear arrangements, as a completely unique alternative. Additionally, epitope-based vaccines have demonstrated various advantages, including safety, the chance to rationally engineer the epitopes for increased potency, breadth, and antigenicity, and therefore the possibility to focus large repertories of immune responses on conserved epitope sequences [16, 17].

2. METHODS

2.1. Selection and antigenic evaluation of Protein

Human Immunodeficiency Virus proteins were selected using the following criteria: (1) reported antigenicity, (2) virulence, and (3) proteins associated with the mechanisms of adhesion. The entire twenty protein sequences were retrieved from Uniprot reference sequence database in FASTA format. For viral protein subcellular localization prediction, CELLO v2.5 was used [18]. The database of GepTop was used to evaluate the essentiality of genes. Proteins were screened by BLASTp to detect sequence homologs to Homo sapiens. To predict transmembrane (TM) helixes, TMHMM v2.0 was used [19]. Compute pI / Mw tool was used to calculate the estimated isoelectric point and relative molecular mass of the amino acid sequences [20].

2.2. Phylogenetic Evolution

Phylogenetic tree of the retrieved sequence of the capsid protein of Human Immunodeficiency Virus was created using Mega X software. 20 of the protein tree was constructed using maximum likelihood parameter within the software.

2.3. T cell Epitope Prediction

To identify MHC-I binding epitopes, NetMHC 4.0 server was used [21]. Fifty human leukocyte antigen (HLA) alleles (HLA-A, -B, -C, and -E) and 6murine alleles (H-2) were evaluated. Predictions were calculated for nine-mers epitopes with a threshold for strong binders of 0.5% and a threshold for weak binders of twenty-two. For MHC-II binding epitopes, NetMHCII 2.3 server; predictions were obtained for 20HLA-DR alleles, 20 HLA-DQ, 9 HLA-DP, and seven mouse H2 class II alleles employing threshold of -99.9, threshold for the strong binder of fifty, and threshold for the weak binder of 20%.

2.4. B cell Epitope Prediction

Linear B cell epitopes of 20-mers were predicted utilizing ABCpred with a threshold of 0.7. The second was BCPred server which was applied with a specificity threshold of 75%. For BepiPred server, only amino acids with score <1.0 were considered for the downstream analysis [22].

2.5. Selection of Epitopes

Epitopes were selected using the following criteria: (1) 20-mer epitopes, (2) epitopes matching on all algorithms, if possible, and (3) potential to bind with the utmost number of MHC-I and MHC-II alleles. For selection, sequences were aligned and overlapped using Clustal Omega server.

2.6. Vaccine Design

Vaxign Server [23] was used to get the vaccine design which shows the protein accession, gene symbol, localization probability, adhesion probability, transmembrane helices and therefore the protein length.

2.7. Protein Prediction and validation of secondary and tertiary structures

The secondary structure of the multi-epitope antigen was predicted using PSIPREDv3.3 [24]. The three-dimensional (3D) structure modeling was performed using Swiss-Model server. Jmol was used for visualizing 3D structures of proteins. For refinement of 3D model structure, Galaxy Refine and Galaxy Loop were applied [25]. The best model was validated by the ProSA web and ERRAT. The residue-by-residue stereo chemical qualities of models were validated by Ramachandran plot obtained from PROCHECK server [26]. The best-refined model was selected.

2.8. Antigenicity, Allergenicity, Solubility, and physicochemical predictions of Vaccine

For antigenicity prediction, VaxiJen server was used. For allergenicity evaluation, AllerTOP v.2.0 was used. For solubility prediction, SOLpro server gotten from Scatch Protein Server was used. Finally, ProtParam was used for the computation of varied physical and chemical parameters [20].

3. RESULTS

TABLE1. THE SUBCELLULAR LOCALIZATION, GENE ESSENTIALITY, VIRULENCE, HUMAN HOMOLOGY, TRANSMEMBRANE HELIX,

Protein	Accession No	Locus Tag	Subcellular Localization	Gene Essentiality	Virulent Protein	Human Homology	TM Helix	pI/MW (kDA)
GAG_HV1H2	sp|P04591|		P – 3.045	ES		N-H	0	9.17 / 55929.86
REV_HV1H3	sp|P69718|		C – 2.232	ES		N-H	0	9.23 / 13050.71
POL_HV1B1	sp|P03366|		P- 1.689	ES		N-H	0	8.93 / 163288.38
POL_HV1N5	sp|P12497|		P – 1.568	N-ES		N-H	0	8.81 / 161788.72
POL_HV2RO	sp|P04584|		C – 2.554	ES		N-H	0	8.70 / 164644.94
POL_HV1H2	sp|P04585|		C – 1.557	ES		N-H	0	8.88 / 162041.90
NEF_HV1BR	sp|P03406|		C – 2.536	ES		H	0	5.85 / 23342.27
ENV_HV1H2	sp|P04578|		OM – 2.128	ES		N-H	3 (21-43aa)	9.17 / 97212.78
ENV_HV1BR	sp|P03377|		OM – 2.332	ES		N-H	2 (21-43aa)	9.03 / 97487.88
VIF_HV1N5	sp|P12504|		P – 2.124	ES		N-H	0	9.93 / 22699.12
VIF_HV1H2	sp|P69723|		P – 2.109	ES		N-H	0	9.89 / 22512.91
VPR_HV1H2	sp|P69726|		C – 3.053	N-ES		N-H	0	8.05 / 11322.81
VPU_HV1BR	sp|P05923|		C – 1.723	N-ES		N-H	1(4-26aa)	4.69 / 9159.72
VPU_HV1H2	sp|P05919|		C – 1.474	ES		N-H	1(5-27aa)	4.69 / 9110.67
VPR_HV1BR	sp|P05928|		C – 3.099	N-ES		N-H	0	7.02 / 11294.76
TAT_HV1H2	sp|P04608|		P – 1.910	N-ES		N-H	0	9.88 / 9837.29
TAT_HV1BR	sp|P04610|		P – 1.874	N-ES		N-H	0	9.88 / 9769.26
ZEP2_MOUSE	sp|Q3UHF7|		E – 1.583	N-ES		H	0	6.49 / 266704.62
ZEP2_HUMAN	sp|P31629|		E – 1.489	ES		H	0	6.50 / 269053.14
ZEP2_RAT	sp|Q00900|		E – 1.551	ES		H	0	6.48 / 267426.14

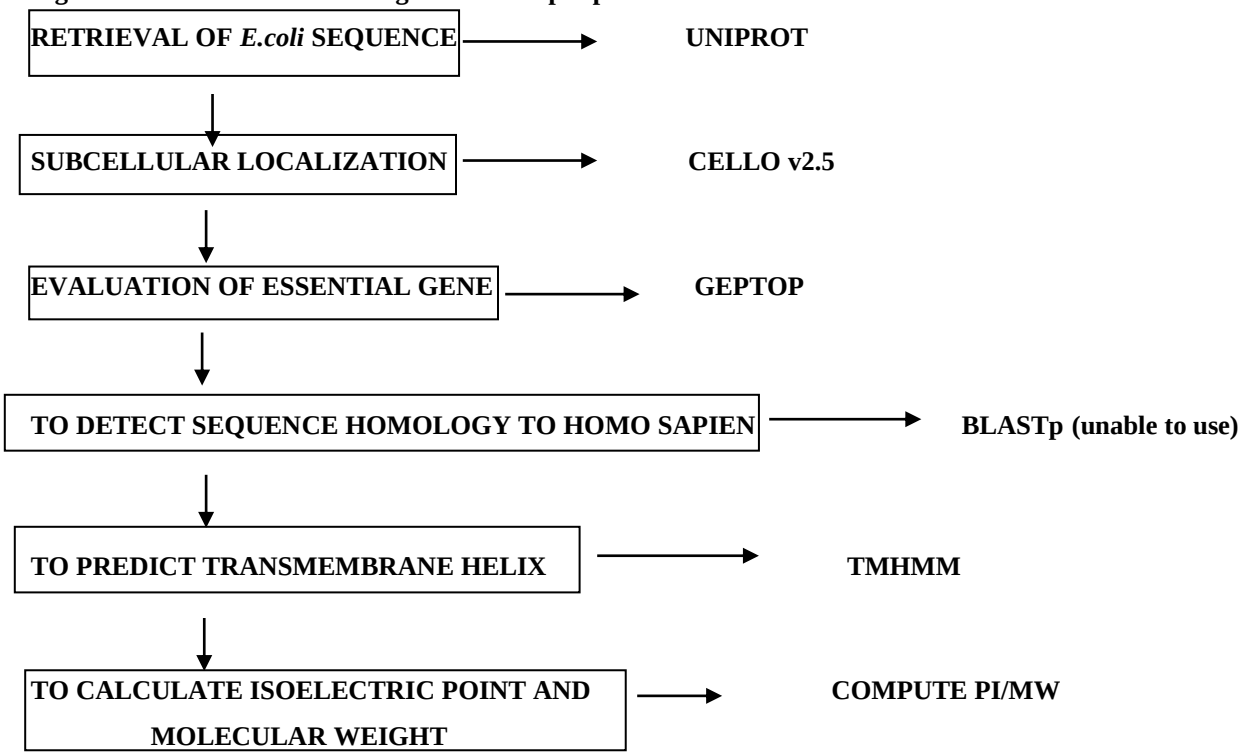
aa, amino acid; C, cytoplasmic; E, essential; ES, extracellular; N-ES, nonessential; N-H, non-homology; N-V, nonvirulent; OM, Outer membrane; P, periplasmic; TM, transmembrane; V, virulent.

TABLE2. POTENTIAL ANTIGENIC EPITOPES PREDICTED BY DIFFERENT SERVERS

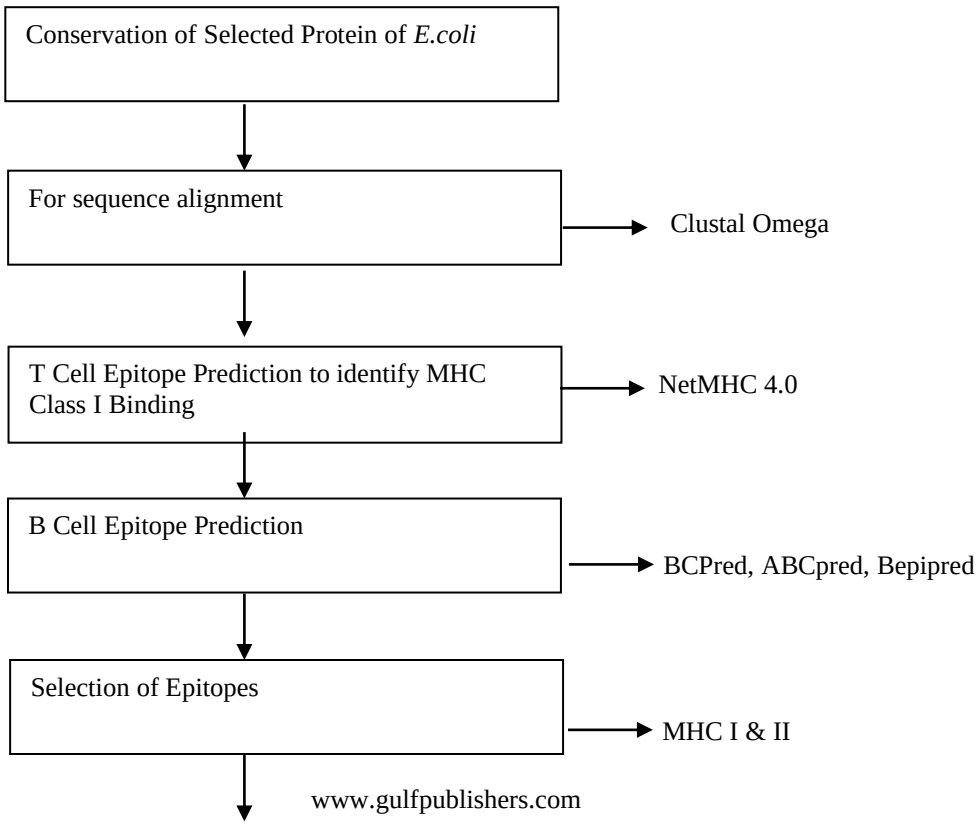
				B CELL EPITOPE			T CELL EPITOPE	
							NetMHC	
ORDER	Protein	Position	Sequence	BCPRED	BepiPred	ABCpred	MHC 1	MHC II
1.	GAG_HV1H2	10-29	GGELDRWEKIRLRPGGKKKY	1.000	0.524	0.650	18H 1M	29H 6M
2.	REV_HV1H3	32-51	EGTRQARRNRRRRWRERQRQ	1.000	0.622	0.830	46H 0M	9H 6M
3.	POL_HV1B1	31-50	LKHIVWASRELERFAVNPGL	0.992	0.489	0.860	26H 0M	35H 4M
4.	POL_HV1N5	10-29	GGELDKWEKIRLRPGGKKQY	1.000	0.514	0.800	10H 1M	19H 6M
5.								
6.	POL_HV2RO	88-107	IHAEEKVKDTEGAKQIVRRH	0.882	0.358	0.850	6H 1M	1H 0M
7.	POL_HV1H2	227-246	QMREPRGSDIAGTTSTLQEQ	1.000	0.549	0.890	19H 1M	20H 0M
8.	NEF_HV1BR	10-29	VVGWPTVRERMRRAEPAADG	1.000	0.521	0.850	26H 0M	30H 10M
9.	ENV_HV1H2	128-147	SLKCTDLKNDTNTNSSSGRM	1.000	0.516	0.820	5H 0M	1H 6M
10.	ENV_HV1BR	136-155	NATNTNSSNTNSSSGEMMME	1.000	0.620	0.940	7H 1M	3H 6M
11.	VIF_HV1N5	67-86	TTYWGLHTGERDWHLGQGVS	0.774	0.563	0.820	17H 1M	7H 5M
12.	VIF_HV1H2	170-189	TEDRWNKPQKTKGHRGSHTM	0.904	0.614	0.660	11H 3M	1H 0M
13.	VPR_HV1H2	2-21	EQAPEDQGPQREPHNEWTLE	0.993	0.605	0.870	12H 3M	1H 0M
14.	VPU_HV1BR	43-62	DRLIERAEDSGNESEGEISA	0.939	0.551	0.880	4H 0M	1H 4M
15.	VPU_HV1H2	22-41	WSIVIIEYRKILRQRKIDRL	0.969	0.535	0.780	28H 2M	12H 12M
16.	VPR_HV1BR	77-96	RHSRIGVTQQRRARNGASRS	0.869	0.749	0.900	10H 0M	13H 2M
17.	TAT_HV1H2	67-86	ASLSKQPTSQPRGDPTGPKE	1.000	0.651	0.760	5H 0M	1H 0M
18.	TAT_HV1BR	45-64	ISYGRKKRRQRRRPPQGSQT	1.000	0.649	0.830	26H 0M	5H 5M
19.	ZEP2_MOUSE	13-32	TSRSGETDSVSGRWRQEQSA	0.916	0.610	0.780	10H 0M	1H 4M
20.	ZEP2_HUMAN	761-780	CRPQLQPGSPSLVSEESPSA	0.997	0.670	0.750	28H 1M	15H 4M
21.	ZEP2_RAT	329-348	IIPKSGIPLPNEGSQYLGPD	0.993	0.599	0.910	21H 4M	2H 0M

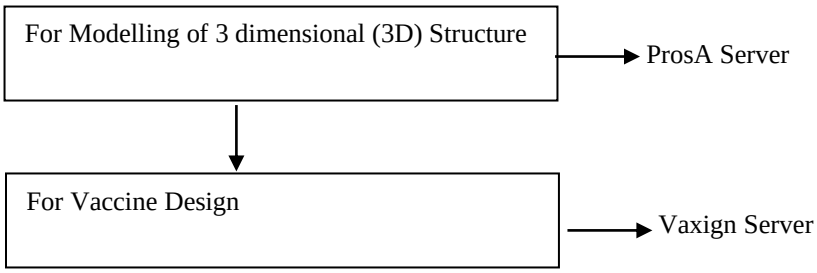
H – human; M - Murine

Figure 1. Flowchart for the Design of Novel Epitope for *E.coli*



FOR CONSERVATION, CONSESUS, SEQUENCE AND ALIGNMENT





ANTIGENICITY, ALLERGENICITY, SOLUBILITY & PHYSICOCHEMICAL PREDICTION OF VACCINE

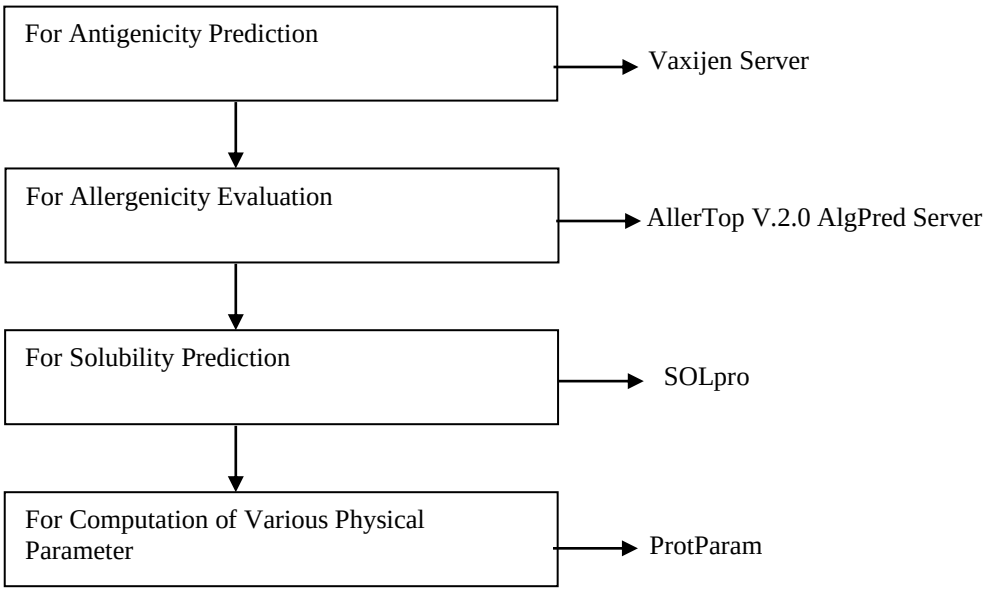


Fig 2: Phylogenetic tree for the selected capsid protein of *HIV* created by Mega X.

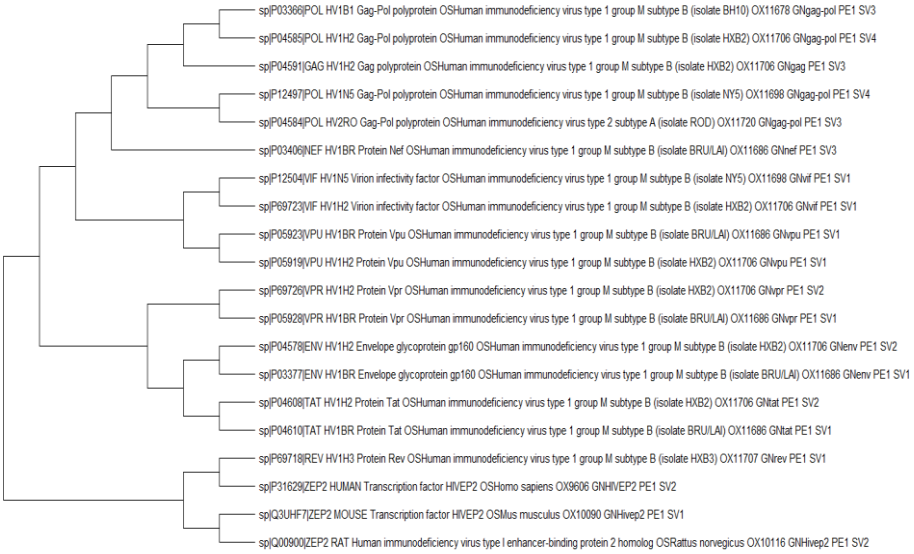


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FIG. 3. The secondary structure prediction of vaccine by PSIPRED. The protein vaccine consists of 27% a helix (H, cylinder), 3.5% b strand (E, arrow), and 69.5% coil (C, line) secondary structural elements. The bar chart represents the percentage of confidence

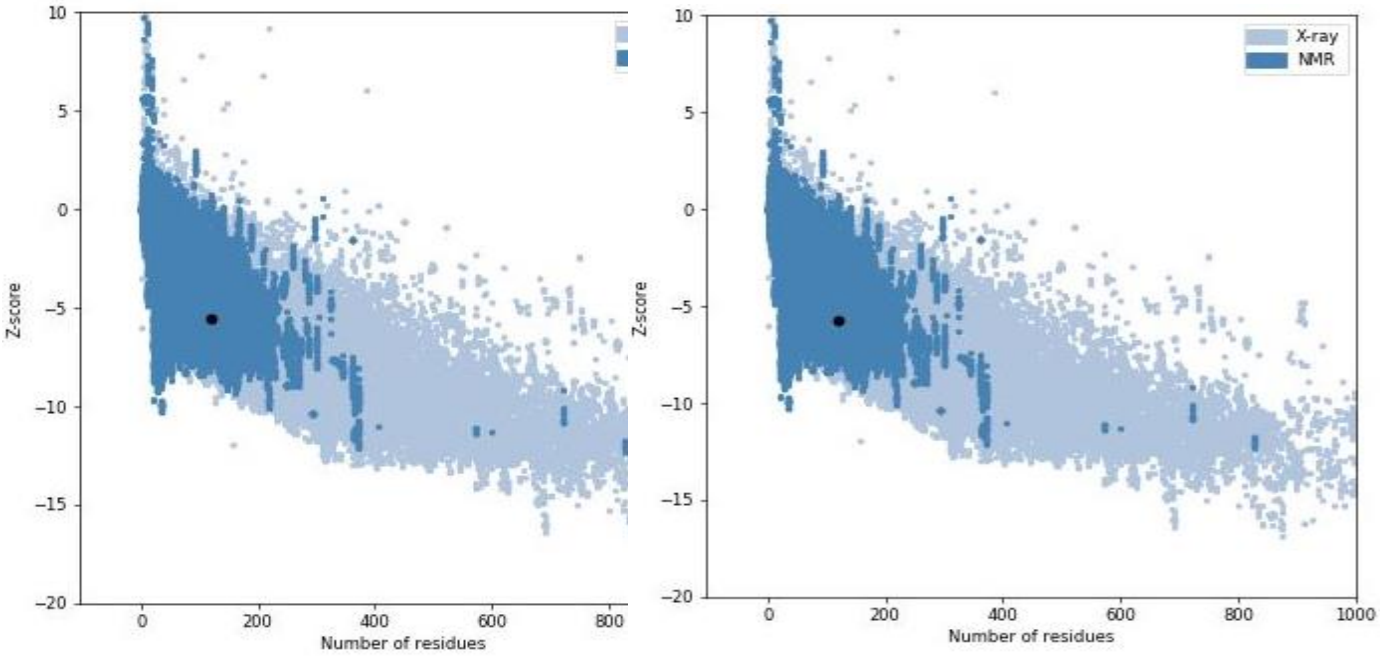


Fig 4: The z-score plot of unrefined and refined 3D structure of vaccine by ProSA-web. (a) The z-score of the starting model is -5.54, (b) The z-score of model after refinement steps is -5.75. The z-scores indicates overall model quality and is depicted as a black spot. The z-scores of all experimentally determined protein chains in current protein data bank (PDB) from NMR spectroscopy (Charcoal) and X-ray crystallography (silver). 3D, three dimensional.

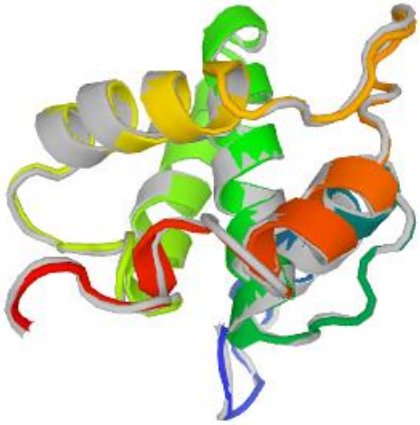
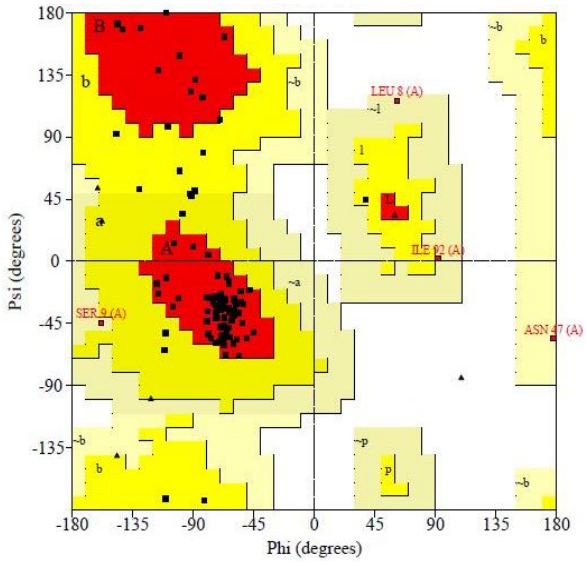


Fig. 5 Comparison of initial and refined 3D protein structure conformation of vaccine by Galaxy Refine.

A



B

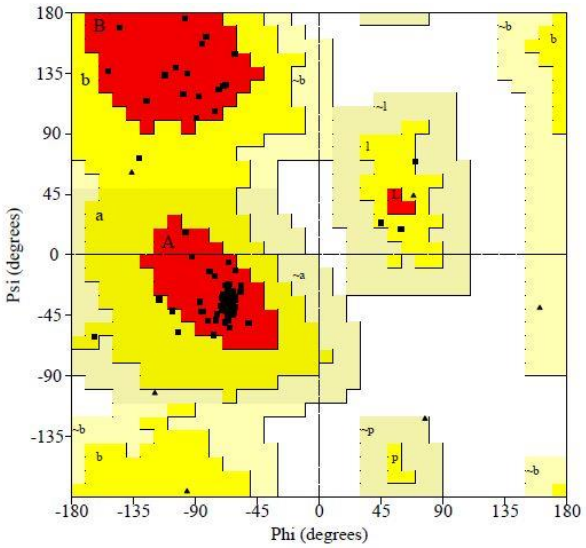


Fig. 6: Validation of vaccine 3D model using Ramachandran plots of (a) the unrefined model and (b) the refined model. The most favored (A, B, and L) and additional allowed (a, b, l and p) regions were demonstrated with charcoal and silver gray colors respectively. The generously allowed regions (-a, -b, -l and -p) are indicated in silver, and the disallowed regions are in white color. Glycine residues are shown in black triangles and other residues of protein are shown in black squares

4.0 DISCUSSION

4.1. Protein Selection and Evaluation

It has been shown that the combination of a long list of antigens can increase the effectiveness of vaccines [27]. A multicomponent vaccine in human volunteers was evaluated; it was observed that the vaccine was safe and highly immunogenic, inducing long-lasting humoral and cellular responses to the antigens. Therefore, 20 proteins were selected for our vaccine.

Selected proteins were further screened in the next step for further parameters, including subcellular localization, essentiality, virulence, non-human homology, TM helixes, and molecular weight. Our predictions showed that REV_HV1H3, POL_HV2RO, POL_HV1H2, NEF_HV1BR, VPR_HV1H2, VPU_HV1BR, VPU_HV1H2, VPR_HV1BR possessed a cytoplasmic location; ZEP2_MOUSE, ZEP2_HUMAN and ZEP2_RAT were extracellular, whereas ENV_HV1H2 and ENV_HV1BR were predicted as outer-membrane proteins. In addition, GAG_HV1H2, POL_HV1B1, POL_HV1N5, VIF_HV1N5, VIF_HV1H2, TAT_HV1H2 and TAT_HV1BR were classified as a periplasmic protein, as shown in Table1. Surface and extracellular proteins are good targets to develop a vaccine aiming toward prevention of viral infections and diseases [28].

In the analysis of essential genes by Geptop, 13 out of the 20 selected protein were predicted to have essential genes. ENV_HV1H2, ENV_HV1BR, GAG_HV1H2, POL_HV1B1, VIF_HV1N5, VIF_HV1H2, ZEP2_HUMAN, ZEP2_RAT, REV_HV1H3, POL_HV2RO, POL_HV1H2, NEF_HV1BR

and VPU_HV1H2 were identified to have essential genes. Essential genes are those genes of an organism that are most important for its survival; essential genes are of particular importance because of their theoretical and practical applications such as studying the robustness of a biological system, defining a minimal genome/organism and identifying effective therapeutic targets in pathogens [29]. Homology analysis of the 20 prioritized proteins using BLASTp revealed <80% identity, which was significant and important to declare the sequences as nonhuman homologs; A good vaccine targets should not be human homologues so as to avoid autoimmunity. The prediction of the topology of proteins by TMHMM showed that VPU_HV1Br and VPU_HV1H2 had one TM helix, ENV_HV1BR had two TM helix and ENV_HV1H2 had three TM helix whereas other proteins do not show the presence of any such topology. VPU_HV1Br and VPU_HV1H2 showed the presence of a TM helix located at 4-26 and at 5-27 amino acid positions, respectively, ENV_HV1BR showed the presence of a TM helix located at 21-43 amino acid position while ENV_HV1H2 showed the presence of a TM helix located at 21-43 amino acid position. Finally, molecular weights calculated by Compute pI/Mw tool of 10 proteins resulted to weigh <50,000 kDa, whereas GAG_HV1H2, POL_HV1B1, POL_HV1N5, POL_HV2RO, POL_HV1H2, ENV_HV1H2, ENV_HV1BR, ZEP2_MOUSE, ZEP2_HUMAN and ZEP2_RAT have molecular weights of 55929.86, 163288.38, 161788.72, 164644.94, 162041.90, 97212.78, 97487.88, 266704.62, 269053.14 and 267426.14 kDa, respectively (Table1).

Good vaccine candidates are considered as those that do not have homology with human proteins to avoid or prevent the generation of a potential autoimmune response; they must also lack TM regions, to facilitate their expression. Another characteristic of a good vaccine candidate is that it should possess good antigenic properties, which are important for the pathogenesis of the microorganism and for protection against the disease [30]. Some authors have used these approaches in the selection of candidate proteins for the insilico design of *Helicobacter pylori* vaccines [31, 32].

4.2. T and B cell epitopes

The prediction analysis by different bioinformatics servers for T and B cells (using MHC-I/-II alleles for human and mouse BALB/c) allowed the selection of 20 epitopes based on their score, number of alleles, and agreement between the servers used. Epitopes obtained were GAG_HV1H2$_{10-29}$, REV_HV1H3$_{32-51}$, POL_HV1B1$_{31-50}$, POL_HV1N5$_{10-29}$, POL_HV2RO$_{88-107}$, POL_HV1H2$_{227-246}$, NEF_HV1BR$_{10-29}$, ENV_HV1H2$_{128-147}$, ENV_HV1BR$_{136-155}$, VIF_HV1N5$_{67-86}$, VIF_HV1H2$_{170-189}$, VPR_HV1H2$_{2-21}$, VPU_HV1BR$_{43-62}$, VPU_HV1H2$_{22-41}$, VPR_HV1BR$_{77-96}$, TAT_HV1H2$_{67-86}$, TAT_HV1BR$_{45-64}$, ZEP2_MOUSE$_{13-32}$, ZEP2_HUMAN$_{761-780}$ and ZEP2_RAT$_{329-348}$.

4.3. Protein Structure Prediction

The vaccine is composed of 400 amino acids, and prediction of secondary structure showed that it contains 15% a helixes, 18% b sheets, and 67% others (random coil and b-turn), as shown in Figure 1.

Five 3D models of protein vaccine were generated among which the model with the highest c-score = 2 was selected for further refinement; the c-score range is typically from 2-3, the higher the value, the higher the confidence.

The quality and potential errors in the best model were analyzed. The initial input model z-score was -5.75, which falls within those commonly observed in similar size-native proteins (Fig. 4A). ProSA-web indicated that the preliminary model requires refinement processes. Hence, the raw model was subjected to loop refinement and energy minimization using galaxy refine. After all refinement procedures, ERRAT factor was improved from 68.8 to 83.3. The z-score of the final model reached a value of -5.54 (Fig. 4B). The starting models was given (Fig. 5).

To validate the 3D models, Ramachandran plot analysis was performed before and after refinement processes. The Ramachandran plots of the unrefined model indicated that 83.0% of residues were located in most-favored regions, 13.2% in the additional allowed region, 3.8% in generously allowed regions, and 0.0% in disallowed regions of the plot (Fig. 6A). The refined model showed that 92.5% of residues were located in most-favored regions, 7.5% in additional allowed regions, 0.0% in generously allowed regions, and only 0.0% in disallowed regions (Fig.6B). Our results indicated that the quality and stability of the final refined model were slightly improved based on Ramachandran plot predictions.

4.4. Antigenicity, Allergenicity, Solubility and physicochemical parameters of the vaccine

An antigenicity score of 0.5277 was obtained. The allergenicity prediction showed that the vaccine is not allergenic. The molecular weight and theoretical pI of protein were 45.6 kDa and 10.36, respectively.

The recombinant protein vaccine solubility upon overexpression in *Escherichia coli* was 0.921491. Half-life was estimated to be 30 hours in mammalian reticulocytes, >20 hours in yeast and >20 hours in *E. coli*. The vaccine was found as stable within stability index of 34.41.GRAVY and aliphatic index were assessed as -1.352 and 51.22 respectively.

5. CONCLUSION

In this study, we designed a novel multi components vaccine against *HIV* based on bioinformatics analysis to produce a huge and robust cellular and humoral immune response for *E. coli* prevention. This novel oral vaccine design could be a good vaccine candidate against *HIV*. However, to make the therapeutic and prophylactic effect of our oral vaccine design valid, *in vitro* and *in vivo* immunological studies will be required.

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