American Journal of Medical and Biological Research, 2019, Vol. 7, No. 1, 12-19
Available online at http://pubs.sciepub.com/ajmbr/7/1/3
Published by Science and Education Publishing
DOI:10.12691/ajmbr-7-1-3

Immunoinformatic Approach for Designing Novel Universal Human Peptide Based Vaccine of Glycoprotein Receptor (M protein) against Rift Valley Fever Virus

Neama Esmat Mahmoud[1,#,*], Marwa Mohamed Osman[2,#], Safa Ibrahim Gebril[3,##], Romaisaa Mubarak Babiker[3,##], Ruaa Mohamed Almardi[3,##], Zainab Abd Elgafour Osman[3,##], Sajda Mergany Silman[3,##], Manasik gumah Adam[3,##], Alsmawal Awad Elimam[1], Zohal AbdalAzeem Bala[1], Tarig Abdalleh Mohammed[1], Ayat Adil Mohammed[1]

[1]Faculty of medical laboratories sciences, Alneelain University- Khartoum, Sudan
[2]Department of Biotechnology, Africa city of Technology- Khartoum, Sudan
[3]Faculty of pharmacy, Khartoum University- Khartoum, Sudan
[#]Contributed equally, [##]Contributed equally.
*Corresponding author: n3oom95@gmail.com

Received August 10, 2019; Revised September 13, 2019; Accepted September 19, 2019

Abstract Rift Valley fever virus (RVFV) is a single strand, negative sense, an envelope spherical particle, of size 80 - 120 nm, segmented RNA virus that belongs to Genus: Phlebovirus of Bunyaviridae family. The clinical manifestations of the disease among animals are abortion and death of newborns. While in humans, although the disease is mild or asymptomatic, there are several reports of high fatality rates. The M segment of RVF virus Genome which encodes the envelope glycoprotein has been used to design a vaccine for immunization against this virus. we aimed to design a novel peptide-based vaccine for RVFV using immunoinformatic approach to predict highly conserved epitopes against glycoprotein receptor" Gn and Gc" of M protein, that can mediate immune response which can use later to produce a new vaccine that could replace the conventional vaccine. A total of 118 sequences of M protein of RVFV were retrieved from NCBI database and stored as FASTA format for immunoinformatics analysis. ClustalW multiple alignment using BioEdit sequence alignment editor (v7.0.9) was performed to the retrieved sequences to identify the conserved region compared to M protein RVFV reference sequence under gene bank accession number [YP_003848705.1]. The B and T cell epitopes prediction is done by immune epitope database (IEDB). (IEDB) predicted B cell epitopes by Bepipred linear epitope prediction analysis and T cell epitopes using Major Histocompatibility Complex class I and ll binding prediction tool based on Stabilized Matrix Method (SMM). Allergenicity for the Helper T cell epitopes (HTL) predicted using AllerTop software. TAP transporter and Proteasomal cleavage for Cytotoxic T cell (CTL) were predicted from (IEDB). The population coverage over the world was determined. The four best predicted CTL namely (^{836}HTYLQSVRK^{844}, ^{672}IPRYSTYLM^{680}, ^{1085}ILHFTVPEV^{1093} and ^{834}FVHTYLQSV^{842}) were docked with HLA-B*35 and suggested to be universal peptide vaccine for immunization against RVFV. The typical overlapping between the MHC Class I epitope (^{834}FVHTYLQSV^{842}) and MHC Class II (^{834}FVHTYLQSV^{842}) suggest the possibility to presenting these antigens to immune system via both MHC class I and II pathways. In conclusion; the four CTL epitopes are selected as vaccine candidates to develop safer and easier to manufacture without need of culture vaccine for prophylactic method against this virus. We recommend to confirm our result by doing additional in vivo and in vitro complementary steps to support this novel predicted vaccine.

Keywords: *rift valley fever virus, immunoinformatic, peptide, vaccine & M protein*

Cite This Article: Neama Esmat Mahmoud, Marwa Mohamed Osman, Safa Ibrahim Gebril, Romaisaa Mubarak Babiker, Ruaa Mohamed Almardi, Zainab Abd Elgafour Osman, Sajda Mergany Silman, Manasik gumah Adam, Alsmawal Awad Elimam, Zohal AbdalAzeem Bala, Tarig Abdalleh Mohammed, and Ayat Adil Mohammed, "Immunoinformatic Approach for Designing Novel Universal Human Peptide Based Vaccine of Glycoprotein Receptor (M protein) Against Rift Valley Fever Virus." *American Journal of Medical and Biological Research*, vol. 7, no. 1 (2019): 12-19. doi: 10.12691/ajmbr-7-1-3.

1. Introduction

Rift Valley fever virus (RVFV) is a single strand, negative sense, an envelope spherical particle, of size 80 - 120 nm, segmented RNA virus that belongs to Genus: Phlebovirus of Bunyaviridae family [1]. It contains three Genomic segments; termed as "L" the larger, "M" medium and "S "small segments. The L encode the RNA-dependent RNA polymerase (RdRp), M segment encodes enveloped glycoprotein (Gn,Gc) and

non-structural protein (NSm), and the S which encode nucleocapsid protein "N" and non- structural protein (NSs) [2,3,4]. The M segment encodes the envelope glycoprotein has been used to design a vaccine for immunization against this virus. The virus is a zoonotic virus primary infect animal (ruminant) such as: cattle, buffalo, sheep, goats and camels and cause disease lead to high mortality and abortion rate in in infected livestock which get infection when bite by infected mosquito, subspecies (Aedes, culex, Anopheles) result in economic loss [3,5,6]. Transmission in humans occurs either from direct transmission from mosquito, or by exposure to infected animal blood, body fluid or tissue, also transmission can be by aerosol during slaughter or maintenance of the infected animal, or by veterinary and obstetric procedures, but there is no human to human transmission has been reported [3,6,7]. This virus has the ability to infect and cause illness in humans. The incubation period take from 2 to 6 days followed by serial of many different diseases, in usual cases the infection is asymptomatic or mild illness that characterized by self-limiting febrile illness, with some exception of about 1-2% of the reported cases develop severe complication, such as hepatitis, encephalitis, retinitis which may lead to blindness, and hemorrhagic fever occurs in less than 1% of cases characterized by profound Coagulopathy, disseminated Intravascular coagulation and multi-organ dysfunction, lead to about 50% mortality rate for those develop hemorrhagic fever, The fatality ratios are 10-20% among hospitalized patients [2,7,8].

RFV virus was disseminated and infected thousands human and animals in many various parts of Africa, as well as Arabian Peninsula with the risk of spreading more to Asian, European and American Region [3]. It first identified in Kenya in the 1930s, and be restricted to the eastern region of Africa until occurred in South Africa in 1951 result in abortion of 500,000 fetus and death of 100,000 animal ,then virus spread to west Africa in 1987s. Between the periods of 1977-1979 it was caused epidemic in Egypt result in massive animal death and 200,000 human infections at died of at least 594 from hospitalized cases were predicted. The infection with a virus was documented for the first time outside Africa in the Saudi Arabia and Yemen. In 2000; about 2,000 human infected and at least 245 deaths. In 2007; RVFV outbreaks have been reported in several eastern African countries, in additional to those African countries; it Affects islands in the Indian Ocean and in Sudan [7,9].

Animal vaccination can prevent death and abortion in livestock and eliminate human infection by controlling one of the main source of human infection. Animal vaccines are available but there are no any commercial available human peptide vaccines for RVFV [5,6]. Formalin-inactivated vaccine for both human and animal are used in endemic country but have drawback of requiring more than vaccine for immunity, use of pathogenic strain, and high cost in manufacturing. The live attenuated vaccine is under development vaccine for both human and veterinary but less effective because the effective vaccine may induce long-Term protective immunity and these vaccines are not induced these long-term Immunities. Another recombinant protein vaccination for ruminant are under development for future controlling of livestock [6,10].

Because that reason for no availability of human peptide vaccine; our study aimed to design universal peptide vaccine for RVFV from the envelope glycoproteins using computational method to predict epitope so including T helper cells mediated immune response, which can be used latter to produce new vaccine that could replace the conventional vaccine depending on *in Silico* approaches and information in databases. We consider this study discriminative because there are no any human peptide-based vaccines for RVFV strain.

2. Material and Method

2.1. Protein Sequence Retrieval

A total of 118 sequences of M protein of RVFV were retrieved from the national center for biotechnology information (NCBI) (https://www.ncbi.nlm.nih.gov/) [10] for immunoinformatics analysis.

2.2. Determination of Conserved Region

Clustal W multiple alignment was performed to the retrieved sequences through BioEdit sequence alignment editor (v7.0.9) [11], to identify the conserved region compared to M protein RVFV reference sequence under gene bank accession number [YP_003848705.1].

2.3. Prediction of Epitopes

B and T cells' epitopes were predicted using The Immune Epitope Data Base (IEDB) (http://www.iedb.org/) [12].

2.3.1. B Cell Epitope Prediction

Immune epitope data base analysis resources (http://tools.iedb.org/bcell/) [13] was used to predict B cell epitopes by Bepipred linear epitope prediction analysis after submitted the reference sequences.

2.3.2. T Cell Epitope Prediction

Cytotoxic T cell epitopes were predicted using Major Histocompatibility Complex class I (MHC class I) binding prediction tool (http://tools.iedb.org/mhci/) [14] based on Stabilized Matrix Method (SMM) [15,16]. Only epitopes that found in conserved region and have IC50 less than 50 was selected and saved for further analysis.

Helper T cell epitopes were predicted using MHC class II prediction tool (http://tools.iedb.org/mhcii/) [17] based on SMM [18]. Only 100% conserved epitope with IC50 <50 were selected and saved for further analysis.

MHC class I (HLA-A and HLA-B) and MHC class II (DR, DP and DQ) alleles were used.

2.4. Tap Score and Proteasomal Score

Tap score and proteasomal score was determined for each selected epitopes by using proteasomal cleavage/TAP transport /MHC class I combined predictor (http://tools.iedb.org/processing/) [19].

2.5. Epitope Allergenicity

Allergic properties of epitopes were determined using AllerTop v.2 (http://www.ddg-pharmfac.net/AllerTOP/) [20]. Only non-allergic epitopes were saved.

2.6. Structural Analysis

2.6.1. Visualization of 3D Structure of M Protein

Three dimension (3D) structure was visualized using UCSF-Chimera visualization tool 1.8 [21] after getting protein data base bank (pdb) of reference sequences by using Raptor-X software (http://raptorx.uchicago.edu/StructurePrediction/predict/) [22].

2.6.2. Molecular Docking

Human allele/receptor (HLA-B*35:1) was chosen according to the study done by Gras S. and his colleagues in 2010 whose reported that, the binding of influenza epitope NP (418-426) to B7 family (HLA-B*3501/03/0702) [23] and other one by Weiskopf D. *et al*. (2013) whose found the B*3501 has high response frequency and magnitude [24]. RVFV epitopes have been selected according to high binding affinity with more than one allele and TAP binding. Their homology modeling in 3D structure has been done with PEP STR MOD-structure prediction of peptide containing natural non-natural and modified residue (http://osddlinux.osdd.net/raghava/pepstrmod/nat_ss.php) [25]. While the HLA-B*35:1 3D structure (PDB:3KLR) that bind with NP(418-426) epitope of influenza virus [23] was retrieved from RCSB protein data bank for docking analysis (http://www.rcsb.org/pdb/home/home.do) [26] and then detecting the influenza ligand using UCSF-chimera [21] before docking applied.

A molecular docking technique was applied using two softwares MTiAutoDock 1.0 [27] and patchDock Beta 1.3 version (http://bioinfo3d.cs.tau.ac.il/PatchDock/) [28] to model the interaction of the 3D-modeled epitopes with human allele. Then visualization has done by the UCSF-chimera visualization tool 1.8 [21] and PYMOL Molecular Graphic system, version 1.8 schrodinger LLC (https://www.pymol.org) [29].

2.7. The Calculation of Population Coverage

The fraction of individuals from the whole world predicted to respond to a given set of epitopes with known MHC restriction was calculated using IEDB population coverage calculation tool (http://tools.iedb.org/tools/population/iedb_input) [30].

3. Result

3.1. Conservancy

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

3.2. Prediction of Cytotoxic T Cell Epitope and Interaction with MHC Class I, Tap Transporter, Proteasomal Cleavage and Modeling

M protein analyzed using IEDB MHC-1 Binding prediction tool to predict Cytotoxic T cell epitope interacted with different types of selected MHC class I alleles in man. Based on consensus SMM method with IC50≤ 50; 24 conserved peptides were predicted to interact with different selected human MHC1 alleles. The 9-mer epitope ^{836}HTYLQSVRK^{844} shows high affinity to interact with (HLA-A*30:01, HLA-A*68:01 and HLA-A*03:01) with IC50 = 35.64, 35.64 and 39.98 respectively. Epitope ^{672}IPRYSTYLM^{680} shows high affinity to interact with (HLA-B*07:02 and HLA-B*35:01) with IC50 of 14.15 and 48.37 respectively, epitope ^{1085}ILHFTVPEV^{1093} shows high affinity to interact with HLA-A*02:03 and HLA-A*02:01 with IC50 of 10.4 and 29.93 respectively, and the epitope ^{834}FVHTYLQSV^{842} shows higher affinity to interact with (HLA-A*02:06 and HLA-A*02:03) alleles with IC50 = 9.52 and 9.53. Results are listed in Table 1 and Figure 1 displays the epitopes at the structural level.

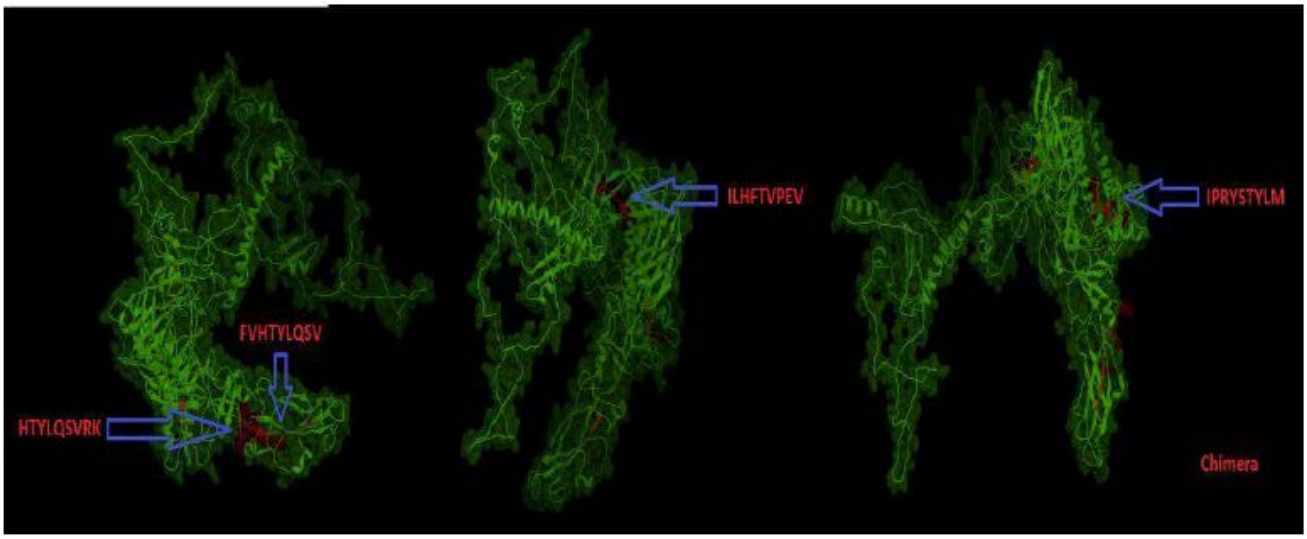

Figure 1. 3D structure of proposed cytotoxic T cell epitopes of M protein of RVF virus, suggested to be interacting with MHC I using (UCSF-Chimera visualization tool). M protein in green color while epitopes in red

Table 1. List of the CTL epitopes which had high binding affinity with the selected Human MHC Class I alleles, Tap transporter and proteasomal cleavage

CTL Epitope	Allele	position	Regio n	length	SMM IC50(nm)	TAP-Score	Proteasome Score
AAVSCDAAF	HLA-B*35:01	1025-1033	G2	9	17.48	1.21	1.16
ASSSRFTNW	HLA-B*58:01	877 -885	G2	9	31.07	0.45	1.33
CLSSRRCHL	HLA-B*08:01	771 - 779	G2	9	30.1	0.45	1.52
EVVPFAVFK	HLA-B*08:01	341 - 349	G1	9	7.25	0.2	1.08
FPLFQSYAH	HLA-B*35:01	200 - 208	G1	9	8.12	-0.35	0.83
*FVHTYLQSV	HLA-A*02:06	834 - 842	G2	9	9.52	0.19	1.08
	HLA-A*02:03				9.53	0.19	1.08
GECHVNRCL	HLA-B*40:01	781 - 789	G2	9	35.47	0.3	1.59
GLMSWFGGP	HLA-A*02:03	1148 - 1156	G2	9	41.78	0.03	0.24
GQSYWTGSF	HLA-B*15:01	759 - 767	G2	9	41.88	0.98	1.22
*HTYLQSVRK	HLA-A*30:01	836 - 844	G2	9	35.64	0.26	1.13
	HLA-A*68:01				39.58	0.26	1.13
	HLA-A*03:01				39.98	0.26	1.13
HVNRCLSWR	HLA-A*31:01	784 - 792	G2	9	18.64	0.62	0.8
	HLA-A*68:01				40.13	0.62	0.8
*ILHFTVPEV	HLA-A*02:03	1085 - 1093	G2	9	10.4	0.15	0.98
	HLA-A*02:01				29.93	0.15	0.98
*IPRYSTYLM	HLA-B*07:02	672 - 680	G1	9	14.15	-0.03	0.93
	HLA-B*35:01				48.37	-0.03	0.93
ITTCSTEGV	HLA-A*68:02	701 - 709	G2	9	25.04	0.18	1.12
KLKTKMKGV	HLA-A*02:03	313 - 321	G1	9	26.79	0.21	1.1
KMWLAATKK	HLA-A*03:01	1187 - 1195	G2	9	36.04	0.4	1
LFQSYAHHR	HLA-A*31:01	202 - 210	G1	9	35.11	0.64	1.14
LMSWFGGPL	HLA-A*02:03	1149 - 1157	G2	9	44.77	0.46	1.14
LTEDCNFCR	HLA-A*68:01	277 - 285	G1	9	42.51	0.5	1.09
MSWFGGPLK	HLA-A*11:01	1150 - 1158	G2	9	43.58	0.34	0.96
MVARVADNI	HLA-A*68:02	635 - 643	G1	9	19.39	0.36	1.19
STAHEVVPF	HLA-A*32:01	337 - 345	G1	9	16.86	1.14	1.22
TVSSELSCR	HLA-A*32:01	749 - 757	G2	9	12.57	0.74	0.96
TYAGACSSF	HLA-A*23:01	183 - 191	G1	9	26.21	1.23	1.22
	HLA-A*24:02				38.96	1.23	1.22

*Proposed epitopes.

3.3. Prediction of Helper T Cell Epitope and Interaction with MHC Class II, Modeling and Allergenicity

There were 14 predicted conserved HTL epitopes found to interact with Human MHC-II alleles by high affinity illustrated in Table 2. The 9-mer peptide (core) ^{834}FVHTYLQSV^{842} showed high affinity to interact with (HLA- DPA1*01:03/DPB1*02:01) with IC50=48 are selected and modeling as a show in Figure 2. These epitopes that bind with different set of MHC Class II alleles by binding affinity ≤ 50 were subjected to AllerTOP 2.0 software to avoid production of IgE antibodies as possible. ^{339}AHEVVPFAV^{347}, ^{833}LFVHTYLQS^{841} and ^{675}YSTYLMLLL^{683} were found to be allergic while ^{834}FVHTYLQSV^{842} was found to be non-allergic.

There were an overlapping of three epitopes from MHC class 1 with that of MHC class 2, ^{341}EVVPFAVFK^{349} overlaps with ^{339}AHEVVPFAV^{347} from MHC class 2, 672IPRYSTYLM680 overlaps with ^{675}YSTYLMLLL^{683} from MHC class 2 while ^{834}FVHTYLQSV^{842} overlaps with two epitopes from MHC class 2 (^{833}LFVHTYLQS^{841} and ^{834}FVHTYLQSV^{842}). These overlapping are illustrated in Table 3.

Table 2. List of the HTL epitopes that had high binding affinity with the Human MHC Class II alleles and Allergenicity using AllerTOP 2.0 software

HTL Epitope (core)	Allele	SMM IC50(nm)	Peptide	position	Region	Allerginicity
LFVHTYLQS	HLA- DPA1*01:03/DPB1*02:01	49	VNPSCLFVHTYLQSV	833 - 841	G2	Probable Allergen
RYSTYLMLL	HLA- DPA1*03:01/DPB1*04:02	35	HAPIPRYSTYLMLLL	674 - 682	G1	Probable non- Allergen
GFKISSAVA	HLA- DQA1*05:01/DQB1*03:01	32	RSTGFKISSAVACAS	497 - 505	G1	Probable non- Allergen
ISSAVACAS	HLA- DQA1*05:01/DQB1*03:01	25	GFKISSAVACASGVC	500 - 508	G1	Probable non- Allergen
FVGAAVSCD	HLA- DQA1*05:01/DQB1*03:01	43	NFEVDFVGAAVSCD A	1022 -1030	G2	Probable non- Allergen
AGACSSFDV	HLA-DRB5*01:01	49	AGACSSFDVLLEKGK	185 - 193	G1	Probable Allergen
AHEVVPFAV	HLA-DRB5*01:01	7	AHEVVPFAVFKNSKK	339 - 347	G1	Probable Allergen
CSELIQASS	HLA-DRB1*01:01	36	ASACSELIQASSRIT	691 - 699	G2	Probable non- Allergen
FKISSAVAC	HLA-DRB1*01:01	37	STGFKISSAVACASG	498 - 506	G1	Probable non- Allergen
FSFIESPGK	HLA-DRB5*01:01	27	NSFSFIESPGKGYAI	903 - 911	G2	Probable Allergen
ISGSNSFSF	HLA-DRB5*01:01	29	ISGSNSFSFIESPGK	897 - 905	G2	Probable non- Allergen
LIQASSRIT	HLA-DRB1*01:01	35	SACSELIQASSRITT	994 - 702	G2	Probable Allergen
*FVHTYLQSV	HLA- DPA1*01:03/DPB1*02:01	48	NPSCLFVHTYLQSVR	834 - 842	G2	Probable non- Allergen
YSTYLMLLL	HLA- DPA1*03:01/DPB1*04:02	31	IPRYSTYLMLLLIVS	675 - 683	G1	Probable Allergen
	HLA- DPA1*02:01/DPB1*01:01	45				
	HLA-DRB1*01:01	18				

*proposed epitope.

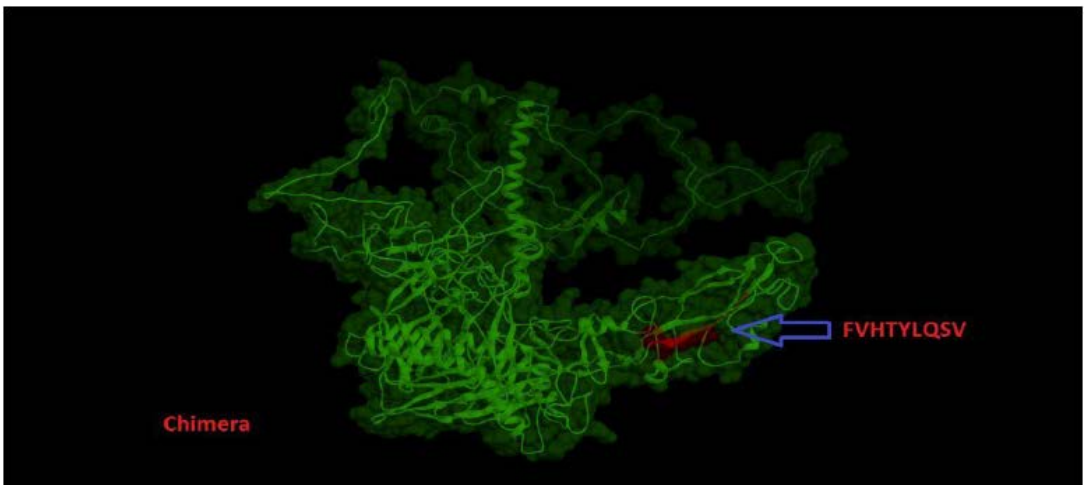

Figure 2. 3D structure of proposed HTL cell epitopes of M protein of RVF virus suggested to be interact with MHC class 2 (UCSF-Chimera visualization tool)

Table 3. Overlapping between MHC class I and II T cell epitopes

CTL Epitope	Peptide	HTL Epitopes	Position	Region
EVVPFAVFK	AHEVVPFAVFKNSKK	AHEVVPFAV	339 – 347	G1
IPRYSTYLM	IPRYSTYLMLLLIVS	YSTYLMLLL	675 – 683	G1
*FVHTYLQSV	VNPSCLFVHTYLQSV	LFVHTYLQS	833 – 841	G2
*FVHTYLQSV	NPSCLFVHTYLQSVR	FVHTYLQSV	834 – 842	G2

*proposed epitope.

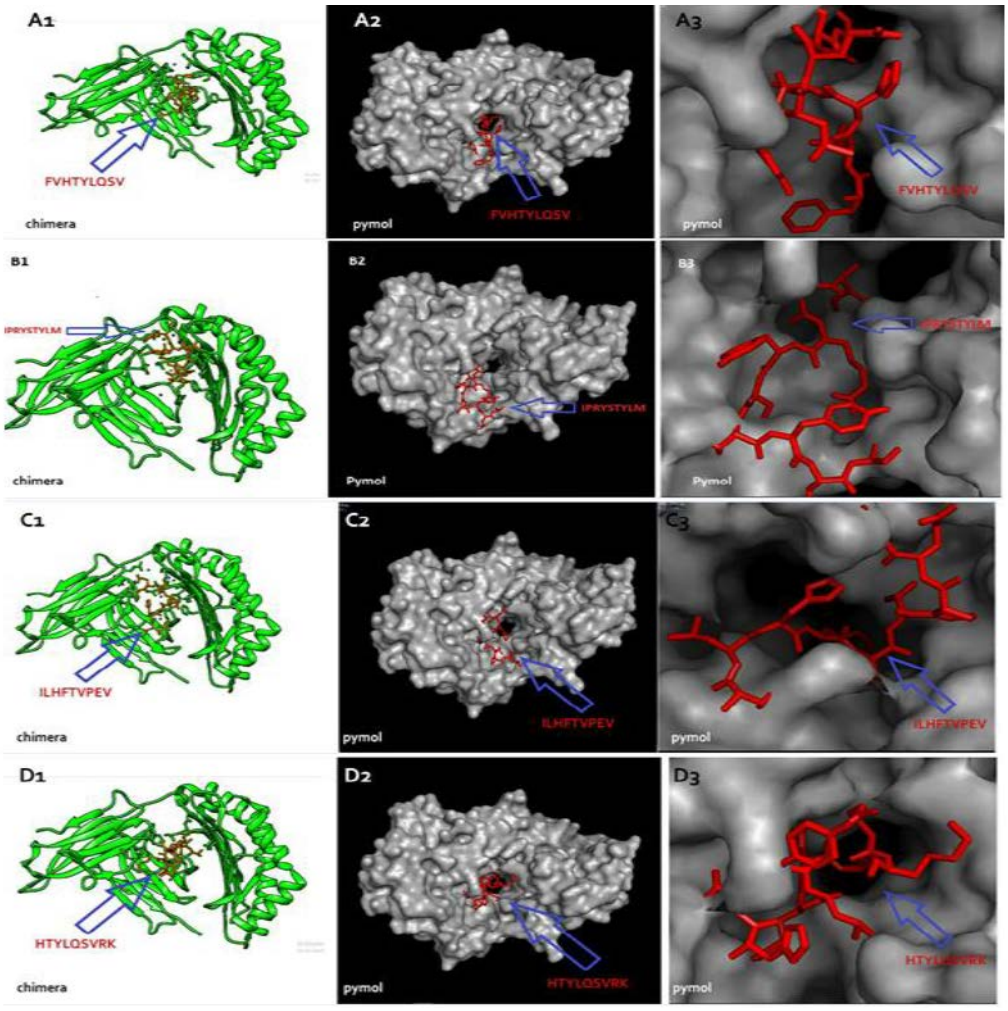

Figure 3. Pictures show the interaction between epitopes and receptors using UCSF-Chimera visualization tool after online docking. Receptors (HLA B*35:01 alleles) represent by green color while CTL Epitopes represent by red one.(2&3) picture show the polar binding interaction between epitopes and receptors after docking, using PyMOL visualization tool (A: ^{834} FVHTYLQSV^{842}, B: ^{672} IPRYSTYLM ^{680}, C: ^{1085}ILHFTVPEV^{1093}, D: ^{836}HTYLQSVRK^{844})

3.4. Molecular Docking of HLA-B*35:01 Allele with Predicted CTL Epitopes

The four predicted CTL peptides that interacted with selected human's MHC-1 alleles: ^{836}HTYLQSVRK^{844}, ^{672}IPRYSTYLM^{680}, ^{1085}ILHFTVPEV^{1093} and ^{834}FVHTYLQSV^{842} were used as "ligands" to detect their interaction with selected HLA (B*35:01) as "receptor" by docking Techniques using offline and online soft wares. Based on polar contacts and the binding energy in kcal/mol unit, the lowest binding energy (kcal/mol) was selected to obtain best binding (pose) and to predict real CTL epitopes as possible, Table 4 and Figure 3.

Table 4. Molecular docking results of proposed epitopes

	MTiAutoDock 1.0	PatchDock
Ligand	**Binding Free Energy (kcal/mol)**	**Scoring**
FVHTYLQSV	-8.47	7854
IPRYSTYLM	-8.7	8384
HTYLQSVRK	-8.72	8130
ILHFTVPEV	-8.71	8126

3.5. Calculation of Population Coverage for MHC 1 and MHC 11

Population coverage analysis to MHC Class I and II which conducted among different 16 geographical areas illustrated in Table 5. The population coverage for MHCI show high percentage in Africa which coverage 68.78% of east African population, 64.97% for central Africa and 81.13%, 80.24%, 73.44% in west, north and south of Africa respectively, while MHCII show low coverage in those area, less than 10%. On the other hand the other part of the world such as Europe, northern America and east Asia show high coverage of MHCI and MHCII, except in central America which show low coverage for both Class I & II.

Table 5. Population coverage for Class I & II proposed epitopes for selected regions

CLASS I		CLASS II	
Population / Area	Coverage	Population/Area	Coverage
East Asia	93.29%	East Asia	10.21%
Northeast Asia	89.93%	Northeast Asia	2.77%
South Asia	82.51%	South Asia	9.25%
Southeast Asia	91.56%	Southeast Asia	0.78%
Southwest Asia	75.39%	Southwest Asia	2.67%
Europe	93.88%	Europe	15.19%
East Africa	68.78%	East Africa	3.96%
Central Africa	64.97%	West Africa	4.47%
West Africa	81.13%	Central Africa	2.12%
North Africa	80.24%	North Africa	2.33%
South Africa	73.44%	South Africa	0.00%
West Indies	89.71%	West Indies	9.55%
North America	92.82%	North America	13.55%
Central America	2.18%	Central America	1.34%
South America	79.71%	South America	4.05%
Oceania	90.28%	Oceania	0.81%

4. Discussion

Peptide vaccine is alternative approach for immunization instead of traditional approach (such as live attenuated and inactivated vaccine) to guarantees safer and easier to produce vaccine without difficulties in manufacture [31]. In our study, we aimed to design universal peptide based vaccine against RVF virus, particularly against glycoprotein receptor" Gn and Gc" using immunoinformatic approach. One type of T-cell called Cytotoxic T (CD8+) play a crucial role in fighting viral infection [31]. Before the peptide recognize by Cytotoxic T cell, peptide undergo number of processes involved in the MHC-I presentation pathway start from cleavage of the large MHC- 1 presented peptide by proteasomes then binding of these peptides with transporter associated with antigen presenting (TAP) and transported to Endoplasmic reticulum, within the Endoplasmic reticulum the peptide with correct size, and proper amino acid sequence bind to MHC-I forming receptor- peptide complex transferred to the surface and recognized by Cytotoxic T lymphocyte [32]. We used stabilized matrix base method (SMM) to predict peptide binding with MHC class I, transporter associated with antigen presenting (TAP) and proteasomal cleavage based on the study done by Peters B. and Sette A. in 2005 [15]. Half-maximum inhibitory concentration (IC50) score which measure the binding affinity of a peptide with MHC-1 is used alternatively to percentile Rank based on the study done by Paul *et al.* in 2013 whose reported the use of IC50 score as the threshold for peptide selection [33]. For TAP transporter we interpreted our result as lower IC50 corresponds to higher TAP binding based on the study done by reported that the amino acid well suited for TAP binding corresponds to low IC50 [34]. We used IEDB Recommended method first because the IEDB software didn't give us Result by SMM

method, then we chose SMM method after uploading the result by delete all other methods and remain SMM method, we selected four conserved epitope which has high binding affinity with (MHC I and TAP transporter) and interacted with more than allele: (^{836}HTYLQSVRK^{844}) interacted with three alleles (HLA-A*30:03, HLA- A*68:01, HLA-A*03:01) and the peptides (^{672}IPRYSTYLM^{680}), (^{1085}ILHFTVPEV^{1093}), (^{834}FVHTYLQSV^{842}), which interacted with alleles (HLA-B*07:02, HLA-B*35:01), (HLA-A*02:03, HLA-A*02:01) and (HLA- A*02:06, HLA-A*02:03) respectively. To suggest the presence of real interaction between CTL epitopes and the selected allele; the four selected peptides were docked with their allele receptor (HLA-B*3501), we used this allele based on two studies, the first study done by Weiskopf D in 2013 reported that B*3501 has high response frequency and magnitude [24], while the second study done in 2010 by Gras S reported the binding of influenza epitope NP (418-426) to B7 family (HLA-B*3501/03/0702) [23]. Because we did not find protein sequence and PDB ID of HLA-B*3501 in NCBI database/structure we obtain PDB ID from crystal structure of HLA-B*3501 that bind to influenza epitope NP (418-426) [23]. The docking result showed that the epitope (^{836}HTYLQSVRK^{844}) has high binding affinity with the binding energy -8.72 kcal/mol and binding energy (-8.7, -8.71, -8.47) kcal/mol with the epitopes (^{672}IPRYSTYLM^{680}), (^{1085}ILHFTVPEV^{1093}), (^{834}FVHTYLQSV^{842}) respectively. Another pathway of immune response against virus is through binding of epitope with MHC Class II to recognize by CD4 T-helper and produce antibody, help in Cytotoxic T lymphocyte activity to fight infected cell, as well as

induce innate immunity by activating phagocytic cell [35]. We found that the epitope (^{834}FVHTYLQSV^{842}) had high affinity to bind with (HLA-DPA1*01:03/DPB1*02:01) allele. Several overlaps observed between MHC class-I and II in T-cell epitope, and that indicate the possibility of antigen presentation to immune system via MHC class I and II [36]. Particularly the epitope (^{834}FVHTYLQSV^{842}) which showed typical overlapping between MHC class I and II and predicted as probable non-allergic when compare with other epitopes (^{339}AHEVVPFAV^{347}, ^{675}YSTYLMLL^{683}, ^{833}LFVHTYLQS^{841}) that had predicted as allergic by allergenicity prediction test. Although the IEDB predicted highly effective T cell epitopes But it shows some limitation because it failed to predict B cell epitopes.

Based on the fact that HLA gene is extremely polymorphic and over thousand different human alleles are known, and those alleles are different through many geographic areas around the world [37]. We conducted population coverage analysis to MHC Class I and II among different 16 geographical areas. The population coverage for MHC I show high percentage in endemic area which coverage 68.78% of east African population, 64.97% for central Africa and 81.13%, 80.24%, 73.44% in west, north and south of Africa respectively, in addition to high coverage in other areas of the world except in central America which show low coverage, about 2.18%. MHCII show low coverage than MHCI, the highest coverage show in areas at risk to developing future outbreak such as Europe coverage 15.19%, then northern America is 13.55% and 10.21% in east Asia, the other parts of the world show less than 10% population coverage.

In Summary: the best predicted epitopes to be Novel human peptide vaccine against M protein of RVFV are: CTL epitopes: ^{836}HTYLQSVRK^{844}, ^{672}IPRYSTYLM^{680}, ^{1085}ILHFTVPEV^{1093} and especially ^{834}FVHTYLQSV^{842} which is also HTL epitope.

5. Conclusion

Our result based on the predictive and analytic tool (IEDB-AR) that usage for prediction of CTL and THL epitopes from protein sequence of glycoprotein coding M protein of RVFV. Then the CTL epitopes are selected as vaccine candidates to develop safer and easier to manufacture without need of culture vaccine for prophylactic method against this virus. We can support our novel universal predicted human vaccine by using further additional in vivo and In vitro complementary steps to confirm our findings.

Acknowledgments

Authors would like to thank Dr. Hisham NourEldayem Altaib Faculty of medical laboratories sciences, Sudan University of science and Technology for his advice.

Competing Interests

The authors declare that they have no competing interests.

References

[1] Kasye, M., Teshome, D., Eshetu, A.A., 2016. A Review on Rift Valley Fever on Animal, Human Health and its Impact on Live Stock Marketing. Austin Virol and Retrovirology 3. Retrieved from www.austinpublishinggroup.com.

[2] Sow, A., Faye, O., Ba, Y., Diallo, D., Fall, G., Faye, O., Sall, A. A., 2016. Widespread Rift Valley Fever Emergence in Senegal in 2013-2014. Open Forum Infectious Diseases, 3(3).

[3] Nanyingi, M. O., Munyua, P., Kiama, S. G., Muchemi, G. M., Thumbi, S. M., Bitek, A. O., Njenga, M. K., 2015. A systematic review of Rift Valley Fever epidemiology 1931-2014. Infection Ecology & Epidemiology, 5(1), 28024.

[4] Mottram, T. J., Li, P., Dietrich, I., Shi, X., Brennan, B., Varjak, M., & Kohl, A., 2017. Mutational analysis of Rift Valley fever phlebovirus nucleocapsid protein indicates novel conserved, functional amino acids. PLoS Neglected Tropical Diseases, 11(12).

[5] Warimwe, G. M., Gesharisha, J., Carr, B. V., Otieno, S., Otingah, K., Wright, D., Hill, A. V. S., 2016. Chimpanzee adenovirus vaccine provides multispecies protection against rift valley fever. Scientific Reports, 6.

[6] Gutiérrez AH, Spero D, Gay C, Zimic M, De Groot AS., 2012. New vaccines needed for pathogens infecting animals and humans: one health. Human vaccines & immunotherapeutics. 8(7): 971-8.

[7] RVF virus facts sheet on National Center for Emerging and Zoonotic Infection Diseases CDC - Division of high consequences pathogens and pathology -2016, available at https://www.cdc.gov/vhf/rvf/.

[8] Soi RK, Rurangirwa FR, McGuire TC, Rwambo PM, DeMartini JC, Crawford TB., 2010 Protection of sheep against Rift Valley fever virus and sheep poxvirus with a recombinant capripoxvirus vaccine. *Clinical and Vaccine Immunology*. 17(12): 1842-9.

[9] M.M., B., D., M., J., V., 2014. Cyclical outbreaks of rift valley fever in East Africa: Why they persist and possible solutions to prevent or contain its spread. American Journal of Tropical Medicine and Hygiene. Retrieved from http://www.ajtmh.org/content/91/5_Suppl_1/228.

[10] Faburay, B., LaBeaud, A. D., McVey, D. S., Wilson, W. C., & Richt, J. A., 2017. Current status of rift valley fever vaccine development. Vaccines. MDPI AG.

[11] Hall, T., Biosciences, I., & Carlsbad, C., 2011. BioEdit: An important software for molecular biology. GERF Bulletin of Biosciences, 2(June), 60-61.

[12] Fleri, W., Paul, S., Dhanda, S. K., Mahajan, S., Xu, X. Peters, B., & Sette, A., 2017. The immune epitope database and analysis resource in epitope discovery and synthetic vaccine design. Frontiers in Immunology. Frontiers Research Foundation.

[13] Larsen JE, Lund O, Nielsen M., 2006. Improved method for predicting linear B-cell epitopes. *Immunome Res.* 2(1): 2.

[14] Lundegaard C, Lund O, Nielsen M., 2008. Accurate approximation method for prediction of class I MHC affinities for peptides of length 8, 10 and 11 using prediction tools trained on 9mers. *Bioinformatics*. 24(11): 1397-8.

[15] Peters B, Sette A., 2005. Generating quantitative models describing the sequence specificity of biological processes with the stabilized matrix method. *BMC bioinformatics.* 6(1): 132.

[16] Morshed MM, Gazi MA, Musa MA, Kibria MG, Uddin MJ, Khan MA, Hasan S., 2014. Computer aided prediction and identification of potential epitopes in the receptor binding domain (RBD) of spike (S) glycoprotein of MERS-CoV. *Bioinformation*. 10(8): 533-38.

[17] Wang P, Sidney J, Dow C, Mothe B, Sette A, Peters B. A., 2008. Systematic assessment of MHC class II peptide binding predictions and evaluation of a consensus approach. *PLoS Comput Biol*. 4(4):e1000048. [PMID:18389056].

[18] Nielsen M, Lundegaard C, Lund O., 2007. Prediction of MHC class II binding affinity using SMM-align, a novel stabilization matrix alignment method. *BMC bioinformatics*. 8(1): 238.

[19] Khan MA, Hossain MU, Rakib-Uz-Zaman SM, Morshed MN., 2015. Epitope-based peptide vaccine design and target site depiction against Ebola viruses: an immunoinformatics study. *Scandinavian journal of immunology*. 2015; 82(1):25-34.

[20] Dimitrov I, Bangov I, Flower DR, Doytchinova I., 2014. AllerTOP v. 2-a server for in silico prediction of allergens. Mol *Model*. 20(6):2278.

[21] Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE., 2004. UCSF Chimera-a visualization system for exploratory research and analysis. *Journal of computational chemistry*. 25(13):1605-12. [PMID: 15264254].

[22] Källberg M, Wang H, Wang S, Peng J, Wang Z, Lu H, Xu J., 2012. Template-based protein structure modeling using the RaptorX web server. *Nature protocols*. 7(8): 1511-22.

[23] Gras S, Kedzierski L, Valkenburg SA, Laurie K, Liu YC, Denholm JT, Richards MJ, Rimmelzwaan GF, Kelso A, Doherty PC, Turner SJ., 2010. Cross-reactive CD8+ T-cell immunity between the pandemic H1N1-2009 and H1N1-1918 influenza A viruses. *Proceedings of the National Academy of Sciences.* 107(28):12599-604.

[24] Weiskopf D, Angelo MA, De Azeredo EL, Sidney J, Greenbaum JA, Fernando AN, *et. al., 2013. Comprehensive analysis of dengue virus-specific responses supports an HLA-linked protective role for CD8+ Tcells. Proceedings of the National Academy of Sciences.* 110(22):E2046-53. [PMID:23580623].

[25] Singh S, Singh H, Tuknait A, Chaudhary K, Singh B, Kumaran S, Raghava GP., 2015. PEPstrMOD: structure prediction of peptides containing natural, non-natural and modified residues. *Biology direct.* 10(1):73.

[26] Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE., 2000. The protein data bank. *Nucleic Acids Res.* 28(1):235-42.

[27] Labbé CM, Rey J, Lagorce D, Vavruša M, Becot J, Sperandio O, Villoutreix BO, Tufféry P, Miteva MA., 2015. MTiOpenScreen: a web server for structure-based virtual screening. *Nucleic Acids Res.* 43(W1):W448-54.

[28] Mashiach E, Schneidman-Duhovny D, Peri A, Shavit Y, Nussinov R, Wolfson HJ., 2010. An integrated suite of fast docking algorithms. *Proteins: Structure, Function, and Bioinformatics.* 78(15):3197-204.

[29] Suvannang N, Nantasenamat C, Isarankura-Na-Ayudhya C, Prachayasittikul V., 2011. Molecular docking of aromatase inhibitors. *Molecules*. 16(5):3597-617.

[30] Bui HH, Sidney J, Dinh K, Southwood S, Newman MJ, Sette A., 2006. Predicting population coverage of Tcell epitope-based diagnostics and vaccines. *BMC bioinformatics*. 7(1): 153. [PMID]:16545123.

[31] World Health Organization. Guide line for production and quality control of synthetic peptide vaccine. WHO technical report series. 1999, No.989.

[32] Singh, S. P., & Mishra, B. N., 2016. Major histocompatibility complex linked databases and prediction tools for designing vaccines. Human Immunology, 77(3), 295-306.

[33] Paul S, Weiskopf D, Angelo MA, Sidney J, Peters B, Sette A., 2013. HLA class I alleles are associated with peptide-binding repertoires of different size, affinity, and immunogenicity. *J Immunol*. 191(12): 5831-9.

[34] Peters B, Bulik S, Tampe R, Van Endert PM, Holzhütter HG. Identifying MHC class I epitopes by predicting the TAP transport efficiency of epitope precursors. *J Immunol*. 2003; 171(4): 1741-9.

[35] Marshall NB, Swain SL., 2011. Cytotoxic CD4 T cells in antiviral immunity. *BioMed Research International*.

[36] Osman MM, ElAmin EE, Al-Nour MY, Alam SS, Adam RS, Ahmed AA, *et.al., 2016.* In Silico Design of Epitope Based Peptide Vaccine against Virulent Strains of (HN)-Newcastle Disease Virus (NDV) in Poultry Species. *Int. J. of Multidisciplinary and Current research*. 4, Available at: http://ijmcr.com/wpcontent/uploads/2016/09/Paper10868-878.pdf.

[37] Mohamed SS, Osman MM, Sami SM, Elgailany HA, Bushara MK, Hassan HA, *et al., 2016.* Immunoinformatics Approach for Designing Epitope-Based Peptides Vaccine of L1 Major Capsid Protein against HPV Type 16. *Int. J. of Multidisciplinary and Current research*. 4, Available at: http://ijmcr.com/wpcontent/uploads/2016/09/Paper11879-896.pdf