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A Novel Multi-Epitope Vaccine For Cross Protection against Hepatitis C Virus (HCV): An Immunoinformatics Approach

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Abstract

Background: Hepatitis C virus (HCV) causes acute and chronic human hepatitis infections. Due to the high genetic diversity and high rates of mutations in the genetic material, so far there has been no approved vaccine against HCV.
Materials and Methods: The aim of this study was to determine conserved B- and T-cell epitopes of E1 and E2 proteins from HCV and to construction a chimeric peptide as a novel epitope-based vaccine for cross-protection against the virus. To this end, one B- and one T-cell epitope from both E1 and E2 which were predicted by EPMLR and Propred-1 server and had the highest score and antigenicity in VaxiJen 2.0 and PAP servers were selected for the construction of chimeric protein as a multi-epitope vaccine.
Results: The results of this study showed that the chimeric peptide had a high antigenicity score and stability. Results also showed that most epitopes of E1 were located in two spectra consist of 45-65, 88-107 and 148-182 while the results of B-cell epitopes of E2 showed that this protein had fewer epitopes than E1. The epitopes predicted for E2 were located in (12-24 and 35-54) spectra.
Conclusion: In conclusion, epitope-based vaccine which was designed by immunoinformatics methods could be considered as a novel and effective vaccine for cross-protection against HCV infection.

Keywords: Hepatitis C virus; Immunoinformatics; Epitope prediction

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Introduction

Hepatitis C virus (HCV) is a RNA virus causes chronic liver disease and a viral pandemic. The HCV was discovered in the USA in 1989 and it is now known that it has infected infects 3% of the world population (1). Acute HCV infection is usually clinically silent and rarely leads to liver failure. Percentage of patients spontaneously clears the virus within 6 months of infection without any treatment. About 55–85% of people with acute infection develops chronic infection and in 15–30% patients is more likely to develop cirrhosis. On the other hand, acute HCV infection is a short-term illness that occurs within the first 6 months after exposure to the HCV. For most people, acute infection leads to chronic infection (2, 3). HCV is usually spread when blood from a person infected with the HCV enters the body of someone who is not infected. Today, most people become infected with the HCV by sharing needles or other equipment to inject drugs (4). Unlike other Hepatitis viruses various details remain unknown about the exact natural processes of HCV, however the key steps of HCV life cycle include: a) Virus attachment to the surface of liver cells; b) Penetration of virus's core protein into the plasma membrane and entrance to the host cell; c) Release of the viral RNA into the host cell; d) The viral RNA then coopts the host cell's ribosomes; e) RNA transcription and creation of new viral RNA; f) Production of protein-based capsomeres from the viral RNA; g) The completed capsomeres assemble around the new viral RNA into new viral particles; and h) The release of new viruses. Because of the genome heterogenicity, lack of small-animal models and inability to grow the virus in cell culture, our knowledge about the HCV lifecycle has been limited (5).
Recently, many studies have been done to introducing

effective vaccines but as yet no approved vaccine for HCV infection has been introduced. In general, there are several approaches to design vaccines against microbial infection including living, inactivated, subunit, toxoids, conjugate, DNA, recombinant vector and peptide vaccines. Fundamental information about the microbe such as life cycle, virulence factor(s) and host cell receptor(s) as well as practical considerations, such as the area where vaccine is used and prevalence play key roles in select the type of vaccine (6). The preparation of a vaccine against HCV needs some basic considerations about the HCV structure and the most important components in its replication. HCV encodes two envelope glycoproteins, E1 and E2 which structure and mode of fusion remain unknown, and so does the virion architecture. The proteins are critical in viral attachment and cell fusion, therefore, studying and studies of these proteins may provide valuable insights into their potential uses in vaccines and antiviral strategies. Many strategies involving the use of mentioned glycoproteins as immunogens are developed for prophylactic vaccination against HCV. Furthermore, neutralizing antibodies targeting epitopes of the E1 and E2 also play a major role in conferring protection against chronic HCV infection and facilitating viral clearance (7, 8). These proteins are two envelope glycoproteins of HCV that play an important role in cell entry. These proteins are highly glycosylated and derived from the N-terminus region of a polyprotein. The E1 acts as the fusogenic subunit and E2 serves as the receptor binding. These proteins are type I transmembrane proteins with a large N-terminal ectodomain facing the endoplasmic reticulum lumen and a C-terminal single membrane spanning transmembrane domain (9-11).

The multi-epitope vaccines are one of the most effective vaccines against different infections. Peptide vaccines are the potential future of vaccination. This type of vaccine is based on synthetic peptides encompassing many B- and T-cell epitopes which can induce specific immune responses.

Computational vaccinology involves the application of computational methods to immunological problems. Prediction of B- and T-cell epitopes has long been the focus of computational vaccinology, and given the potential translational implications, many bioinformatics tools have been developed (12, 13). This study was planned to predict linear B and T-cell epitopes of E1 and E2 envelope glycoproteins of HCV in order to introduce a chimeric multi-epitope peptide as a new candidate for vaccine development against HCV.

Materials and methods

Retrieval amino acid sequences and homology analysis

Amino acid sequences of E1 and E2 proteins of HCV with 192 and 73 amino acids in length respectively, were retrieved from NCBI (http://www.ncbi.nlm.nih.gov/protein/) with accession numbers of ACN92051.1 and AAX36013.1, respectively. Then these proteins were subjected to the Basic Local Alignment Search Tool (BLAST) against the non-redundant protein database available at Uni-prot (www.uniprot.org/). Also for determination of conserved area in the proteins multiple sequence alignment was used between different sequences with the highest score and identity.

Table1. Results of linear B-cell epitope prediction of E1 and E2 proteins of HCV.

Protein	Rank	Epitope sequence	Score	Location
E1	1	MCGAVFLVGQ AFTFRPRRH	1	89-107
	2	CWTPVTPTVAV RYVGATTA	1	47-65
	3	RCWTPVTPTVA VRYVGATT	1	46-64
	4	DMCGAVFLVG QAFTFRPRR	1	88-106
	5	NWTPAVGMVV AQVLRLPQS	1	134-152
	6	ADDVILHTPGCI PCVQDGN	0.8519	25-43
	7	QTCNCSLYPGH LSGHRMAW	0.79149	111-129
	8	EADDVILHTPG CIPCVQDG	0.67783	24-42
	9	VQTCNCSLYPG HLSGHRMA	0.57764	110-128
	10	MNWTPAVGMV VAQVLRLPQ	0.57103	133-151
	11	GAHWGMLAGL AYYSMEGNW	0.50305	159-177
	12	GGAHWGMLAG LAYYSMEGN	0.36484	158-178
	13	ASVRSHVDLLV GAATMCSA	0.21354	65-83
E2	1	NTRTVGGQIAR QLQPFTRL	1	36-54
	2	HNTRTVGGQIA RQLQPFTR	1	35-53
	3	LFSVGPNQNIQ LINTNGSW	-0.13	54-72

B-cell epitope prediction

For prediction of linear B-cell epitopes, the full-length sequence of E1 and E2 were subjected to EPMLR analysis with overall sensitivity of 81.8% at

(http://www.bioinfo.tsinghua.edu.cn/epitope/EPMLR/). The cutoff score >-0.15 was selected for all predicted B-cell epitopes and 19mer epitope was selected for prediction.

Table 2. Results of T-cell epitope prediction of E1 and E2 proteins of HCV.

Protein	Predicted epitope	Amino acid positions	ProPred1 Log scores
E1	DCPNSSIVY	15	0.9163
	EADDVILHT	24	0.9163
	VTPTVAVRY	51	0.9163
	VGDMCGAVF	86	0.9163
	RLPQSIFDI	148	4.2042
	MKMNWTPAV	131	3.7029
	DMCGAVFLV	88	3.3649
	IIMVMVSGV	182	2.9639
	RLPQSIFDI	148	4.9357
	IIMVMVSGV	182	4.9298
	ILGGAHWGM	156	4.1485
	DMCGAVFLV	88	3.9172
	MVVAQVLRL	141	3.8628
	YVGDMCGAV	85	3.5835
	KVGIIMVMV	179	3.1781
	IIMVMVSGV	182	3.0155
E2	RTVGGQIAR	38	0.2231
	WGVMFGLAY	2	-0.4700
	LLLTAGVDA	24	4.2219
	RQLQPFTRL	46	4.0489
	QPFTRLFSV	49	3.2881
	KVIVILLLT	19	2.4849
	SMQGAWAKV	12	4.4473
	VILLLTAGV	22	4.0969

T-cell epitope prediction

T-cell epitope prediction was performed using Propred-1 with sensitivity and specificity of 4% as default threshold (47 MHC Class-I alleles) utilizing amino acid position coefficients. This tool was used to identify common epitopes that bind to both the MHC class molecules as well as to count the total numbers of interacting MHC alleles.

Antigenicity of predicted epitopes

Antigenicity of both B- and T-cell epitopes predicted by the tools were determined using VaxiJen 2.0 server with prediction accuracy of 70% to 89% (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) with 0.4 threshold and Predicting Antigenic Peptides (PAP) server (http://imed.med.ucm.es/Tools/antigenic.pl). Then antigenicity plot of the evaluated proteins was created by PAP based on their sequences.

Allergenicity prediction and IgE epitopes

One of the most important notes about protein and peptide vaccines is that these molecules should not have allergenicity. Therefore, the chimeric peptide was checked for allergenicity by AlgPred server (http://www.imtech.res.in/raghava/algpred/).

Construction of chimeric peptide

In this section, more probable epitopes with the highest score and antigenicity were selected for construction of chimeric peptide as a multi-epitope vaccine. For this, one B- and one T-cell epitope from both E1 and E2 were selected for construction of chimeric peptide.

Table 3. Results of antigenicity determination of predicted B-cell epitope by VaxiJen and PAP servers.

Protein	Rank	Epitope sequence	VaxiJen score	PAP score
E1	1	MCGAVFLVGQ AFTFRPRRH	0.4101	1.0561
	2	CWTPVTPTVAV RYVGATTA	0.9165	1.0859
	3	RCWTPVTPTVA VRYVGATT	1.1220	1.0839
	4	DMCGAVFLVG QAFTFRPRR	0.4122	1.0557
	5	NWTPAVGMVV AQVLRLPQS	0.1183	1.0816
	6	ADDVILHTPGCI PCVQDGN	0.1745	1.0951
	7	QTCNCSLYPGH LSGHRMAW	0.1367	1.0552
	8	EADDVILHTPG CIPCVQDG	0.0659	1.0938
	9	VQTCNCSLYPG HLSGHRMA	0.0166	1.0729
	10	MNWTPAVGMV VAQVLRLPQ	0.4771	1.0711
	11	GAHWGMLAGL AYYSMEGNW	1.0461	0.9888
	12	GGAHWGMLAG LAYYSMEGN	0.6814	0.9943
	13	ASVRSHVDLLV GAATMCSA	0.3377	1.0968
E2	1	NTRTVGGQIAR QLQPFTRL	0.3304	0.9955
	2	HNTRTVGGQIA RQLQPFTR	0.4465	1.0084
	3	LFSVGPNQNIQ LINTNGSW	0.9172	1.0087

Amino acid composition and physicochemical properties

Amino acid composition and some physicochemical properties of constructed peptide which consisted of: Size, Molecular weight, Iso-electric point, Extinction coefficient, Net charge at pH 7, Estimated solubility in water, Estimated half-life in the mammalian reticulocytes, Instability index, Aliphatic index, Hydropathy and amphipathicity were determined using Protparam (http://web.expasy.org/cgi-bin/protparam/protparam), Pepcalc (http://pepcalc.com/), TCDB server (http://www.tcdb.org/progs/?Tool=hydro), and Calctool (http://www.calctool.org/).

Table 4. Results of antigenicity determination of predicted T-cell epitope by VaxiJen and PAP servers.

Protein	PredictedT-cell epitope	VaxiJen score	PAP score
E1	DCPNSSIVY	0.3583	1.0846
	EADDVILHT	-0.2127	1.0671
	VTPTVAVRY	1.1828	1.1210
	VGDMCGAVF	-1.0252	1.0853
	RLPQSIFDI	-0.1844	1.0404
	MKMNWTPAV	2.2262	0.9110
	DMCGAVFLV	-0.5748	1.0958
	IIMVMVSGV	0.0685	1.0760
	ILGGAHWGM	0.8801	1.0108
	MVVAQVLRL	0.2335	1.1471
	KVGIIMVMV	0.8207	1.0657
	RTVGGQIAR	0.9616	1.0180
	WGVMFGLAY	0.6455	1.0319
	LLLTAGVDA	0.2078	1.1058
	RQLQPFTRL	0.4881	1.0112
	QPFTRLFSV	-0.5974	1.0381
E2	VILLLTAGV	0.5098	1.1415
	SMQGAWAKV	-0.0165	0.9597
	KVIVILLLT	0.5846	1.2188

Secondary and 3D structure prediction

The secondary structure of constructed peptide was predicted by GORIV (http://cib.cf.ocha.ac.jp/bitool/GOR/GOR.php). Also, 3D structure of the chimeric peptide was generated using Swiss model server (https://swissmodel.expasy.org).

Evaluation of model stability and solvent accessibility

Themodelstabilityandstructuralqualityof constructed peptide were validated based on Ramachandran plot using PROCHECK server (http://services.mbi.ucla.edu/PROCHECK/). Solvent accessibility is one of the determining factors in the antigenicity of peptide and protein vaccines. Therefore, the constructed peptide was validated in terms of solvent accessibility using SARpred server (http://www.imtech.res.in/raghava/sarpred/).

Molecular docking study

In order to validate the binding affinity of the chimeric peptide to MHCI (with PDB entry 1HLA) and MHCII (with PDB entry 1DLH), molecular docking study was carried out by HEX 6.0 software. The selected parameters for docking study were FFT Mode–3D fast life, Distance Range – 40, Twist range – 360, Correlation type –Shape only, Grid Dimension – 0.6, Receptor range – 180 and Ligand Range –180.

Results

Sequence Retrieval and Homology Analysis

The BLAST of E1 and E2 amino acid sequences revealed 88-99% and 66-78% similarity between E1 and E2 among the different subtypes of HCV, respectively. The results of multiple sequence alignments of E1 and E2 residues showed high degree of conservation among different subtypes of HCV.

B-cell epitope prediction

The results of B-cell epitope prediction of E1 and E2 proteins are shown in Table1. The results indicated that E1 had more B-cell epitopes than E2. Most epitopes of E1 were located in two spectra of 88-107 and 45-65. The predicted epitopes in mentioned spectra had the highest score and could be the main epitopes for humoral immune stimulation.

Unlike E1, the results of B-cell epitopes of E2 showed that this protein had fewer epitopes than E1. Most epitopes predicted for E2 were located in 35-54 spectra so this range in E2 similar to E1 could be considered as stimulating the humoral response of the immune system.

T-cell epitope prediction

The results of T-cell epitopes prediction of E1 and E2 proteins are presented in Table 2. The results showed that these two proteins have multiple T-cell epitopes with high scores. E1 had three predicted epitopes consisted of RLPQSIFDI, IIMVMVSGV, and ILG GAHWGM with the highest score that was located in 148-182 spectra. On the other hand, unlike E1, E2 had fewer T-cell epitopes than E1 and in general the predicted epitope for E2 had a lower score.

However, three epitopes consisted of (SMQGAWA KV, VILLLTAGV, and LLLTAGVD A) were the most probable epitopes of E2 that were located in 12-24 spectra. Further comparative studies of E1 and E2

showed that although the number of epitopes predicted for E1 was more than E2, E2 was more immunogenic due to the number of epitopes on the total number of amino acids.

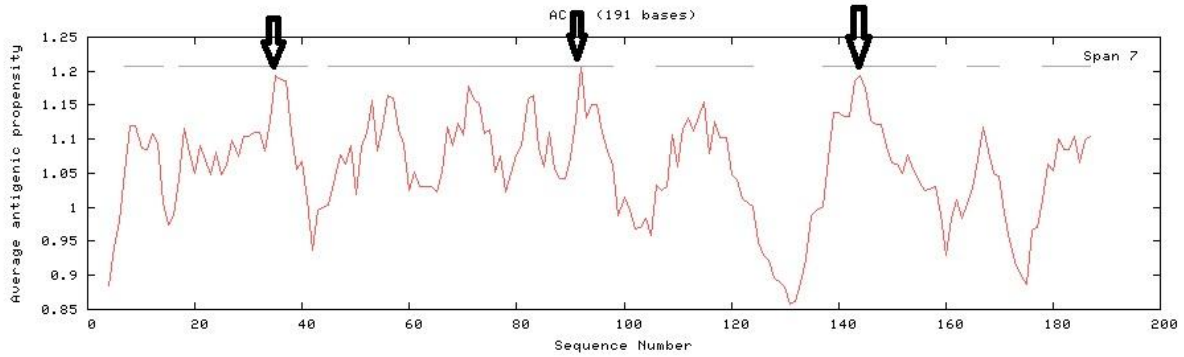

Figure 1. Antigenic plot of E1.

Antigenicity of predicted epitopes

To ensure the results of B- and T-cell epitope prediction, antigenicity of predicted epitopes was investigated by VaxiJen and PAP servers. The results of antigenicity of predicted B- and T-cell epitopes are given in Tables 3 and 4, respectively. Based on the results, it was found that most B- and T-cell epitopes that had a high score in Propred and EPMLR received high marks in terms of antigenicity.

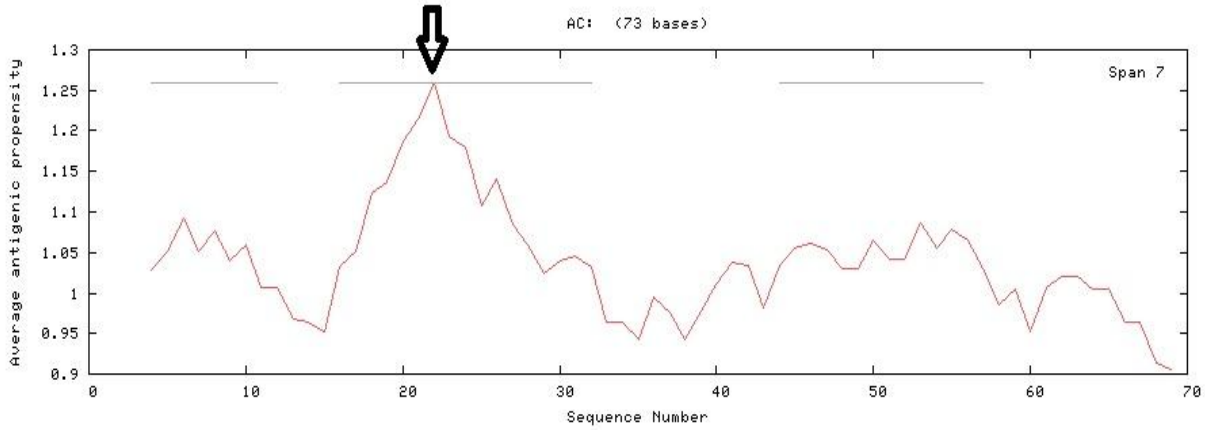

Figure 2. Antigenic plot of E2.

Also, it became clear that two B-cell epitopes consists of (RCWTPVTPTVAVRYVGATT and HNTRTVG GQIARQLQPFTR) were the most antigenic epitopes of E1 and E2, respectively. In this regard, it was found that the most antigenic T-cell epitopes of E1 and E2 were MKMNWTPAV and RTVGGQIAR, respectively.

Table 5. Physicochemical properties of E1 and E2 proteins of HCV (MW is Molecular weight, Ii: Instability index, Ai: Aliphatic index and Ec is Extinction coefficient)

Protein	Size (nm)	PI	Net-charge	MW (Da)	Solubility	Half-life (h)	Ii	Ai	Ec ($M^{-1}cm^{-1}$)
E1	38.4903	6.63	-0.9	20830.01	poor	5.5	21.7	90.31	43100
E2	28.0062	11.18	3.3	8024.13	poor	3.5	21.46	96.16	18350
Chimeric	25.4818	11.83	5.1	6044.03	poor	1	32.13	100.89	12660

The antigenic plot of E1 and E2 are shown in Figures1 and 2, respectively.These plots represent the average of antigenicity of proteins based on the sequences.The antigenicity plot of E1 and E2 showed that three positions in E1 and one position in E2 had highantigenicity. These positions were in accordance with the core of the predicted epitopes with high score and antigenicity.

Construction of chimeric peptide

As it was mentioned, a peptide that was composed by more probable B- and T cell epitopes with the highest antigenicity and the one located in more conserved area of E1 and E2 sequence was introduced as a candidate vaccine. This peptide composed of 56 amino acids with the following sequence: RCWT PVTPTVAVRYVGATTHNTRTVGGQIARQLQPF TR ILGGAHWGM VILLLTAGV.

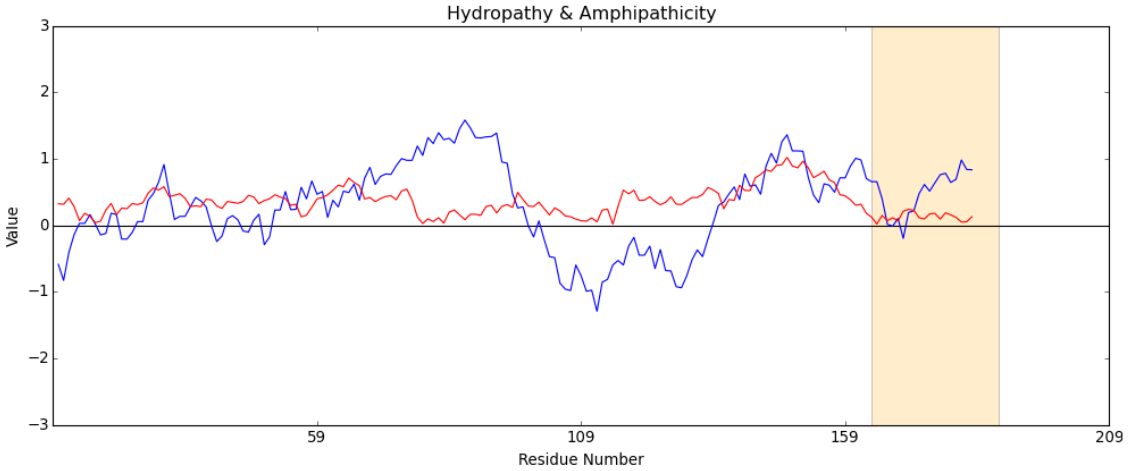

Figure 3. Hydropathy and amphipathicity plot of E1 protein of HCV

Amino acid composition and physicochemical properties

The results of physicochemical properties of E1, E2 and chimeric peptide are shown in Table 5. Results of this section indicated that all three proteins had poor water solubility. Also in term of electrostatic charge, E1 had negative net charge while this parameter was determined as +3.3 and +5.1 for E2 and chimeric peptide, respectively.

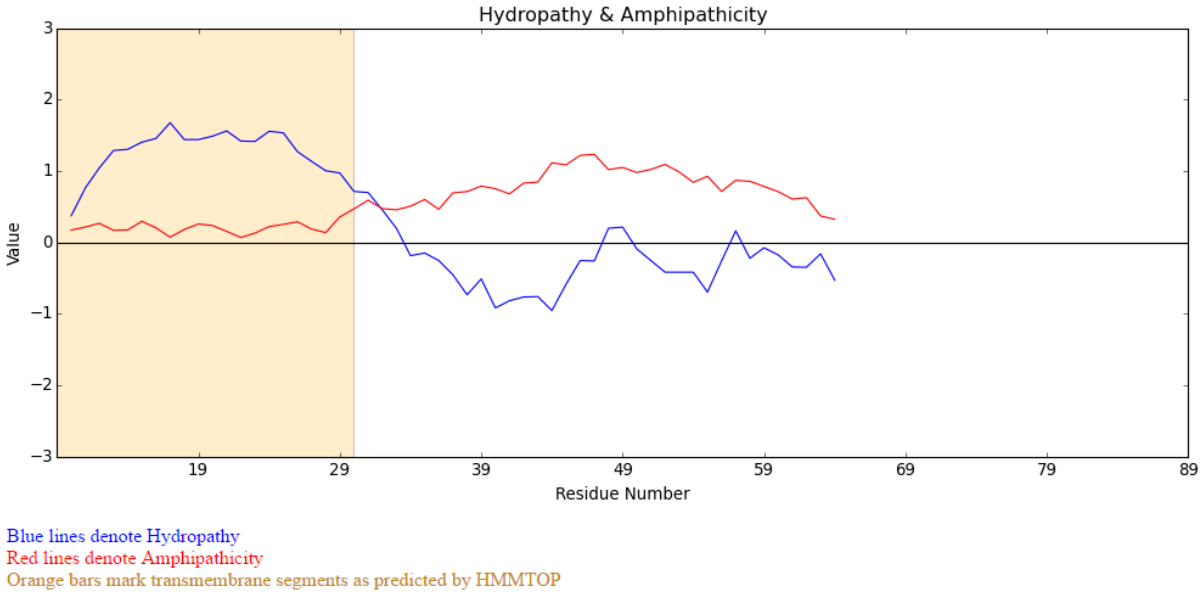

Figure 4. Hydropathy and amphipathicity plot of E2 protein of HCV.

The hydropathy and amphipathicity plot of mentioned proteins are shown in Figures 3-5 respectively. These plots indicated that unlike E1 and E2 proteins, the chimeric peptide was more amphiphilic; therefore, it's transfer in body fluids in free mode and the use of vaccine carrier could be more effective.

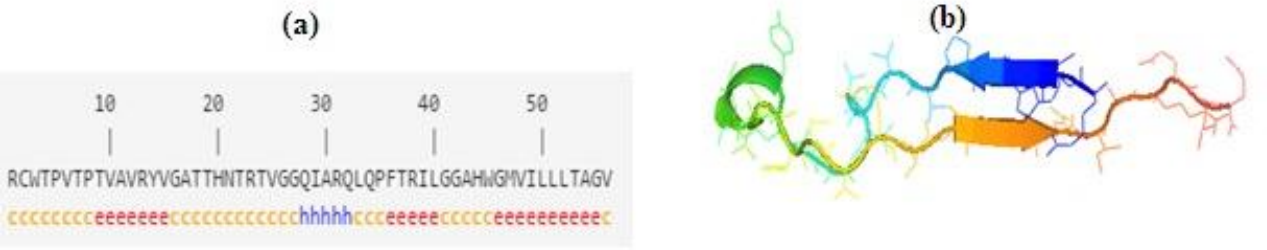

Figure 6. Secondary (a) and 3D structures (b) of chimeric peptide.

Secondary and 3D structure prediction

The secondary and 3D structure prediction of chimeric peptide are illustrated in Figure 6 (a, b) respectively. The results indicated that the percentage of random coils (51.79 %) was more than that of beta-sheets (39.29 %) and alpha-helices (8.93%). Also, tertiary structures of chimeric peptide modeled by Swiss model based on homology (similarity) with the N-terminal domain of HCV envelope glycoprotein E1 as the template, showed that this peptide organized in two beta strands, one short helix, and one long coil.

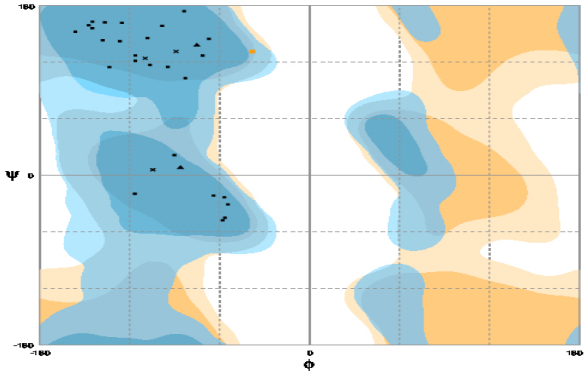

Figure 7. The Ramachandran plot of the chimeric protein. Percentage of the residue was 96.9 % in the favored region, 10 % allowed and without outlier.

Evaluation of model stability and Solvent accessibility

Ramachandran plot of the chimeric peptide is depicted in Figure 7. The results revealed that 96.9% of amino acid residues from modeled structure generated by Swiss model incorporated in the favored regions of the plot. Results also showed that 3.1% of residues were located in allowed regions while there was no amino acid in the outlier region. The results of solvent accessibility prediction are presented in Table 6. Results showed that chimeric peptide has high level of solvent accessibility, especially in the central area. In addition, it was found that certain amino acids such as Arg1 (58%), Cys2 (48%), Gly55 (39%), Val56 (36%), and Val6 (34%) gave a high relative solvent accessibility when compared to other amino acids in the chimeric construct.

Allergenicity prediction and IgE epitopes

The result of allergenicity prediction of chimeric peptide revealed that this peptide did not contain IgE epitope and was considered as non-allergen. Allergenic potential of mentioned peptide was determined -0.5 (threshold was -0.4). Furthermore, positive and negative predictive values were 18.21% and 71.24%, respectively.

Molecular docking studies

The results of molecular docking study confirmed that chimeric peptide had high affinity to both MHCI and MHC II. Results revealed that chimeric peptide had more affinity to MHCII with e-value of -100.3 while e-value was determined -90.7 for MHCI.

Discussion

The results of the study revealed that designed chimeric peptide composed of conserved B- and T-cell epitopes of E1 and E2 protein of HCV had a high level of antigenicity and affinity to MHC I and MHCII. So, it can be considered as a novel vaccine for cross-protection against HCV infection. In recent years, great efforts have been done to introduce effective vaccine against HCV infection. In this regard Kumar *et al.* introduced new hepatitis C virus-like particles (HCV-LPs) encoding structural proteins for genotype 3a. This vaccine was designed for Indian patients and was not effective against other serotypes(14). Zhu *et al.* constructed novel adeno-associated virus (AAV) vectors expressing the full-length NS3 or NS3/4 protein of HCV genotype 1b. This candidate vaccine could well stimulate the immune system but was limited to a specific serotype (15). However, due to the high genetic diversity and high rates of mutations in the genetic material, so far there is no approved vaccine against HCV.

Immunoinformatics as a branch of bioinformatics is a new approach with a variety of tools and databases for *in silico* analysis and modeling of immunological data. One of the major applications of

immunoinformatics that in recent years has been highly regarded is the design of epitope-based vaccines (16-22).

Table 6. Results of solvent accessibility prediction for chimeric peptide.

Number of residue	Residue	Relative solvent accessibility	% Relative solvent accessibility
1	R	0.58	58
2	C	0.48	48
3	W	0.16	16
4	T	0.29	29
5	P	0.29	29
6	V	0.34	34
7	T	0.33	33
8	P	0.28	28
9	T	0.17	17
10	V	0.19	19
11	A	0.11	11
12	V	0.15	15
13	R	0.12	12
14	Y	0.16	16
15	V	0.19	19
16	G	0.22	22
17	A	0.19	19
18	T	0.23	23
19	T	0.17	17
20	H	0.18	18
21	N	0.14	14
22	T	0.19	19
23	R	0.24	24
24	T	0.17	17
25	V	0.15	15
26	G	0.20	20
27	G	0.18	18
28	Q	0.21	21
29	I	0.18	18
30	A	0.18	18
31	R	0.23	23
32	Q	0.29	29
33	L	0.17	17
34	Q	0.20	20
35	P	0.28	28
36	F	0.23	23
37	T	0.20	20
38	R	0.18	18
39	I	0.28	28
40	L	0.21	21
41	G	0.19	19
42	G	0.20	20
43	A	0.18	18
44	H	0.24	24
45	W	0.13	13
46	G	0.17	17
47	M	0.13	13
48	V	0.07	7
49	I	0.10	10
50	L	0.10	10
51	L	0.17	17
52	L	0.17	17
53	T	0.20	20
54	A	0.25	25
55	G	0.39	39
56	V	0.36	36

Although Immunoinformatics-based vaccine design is able to achieve cost-efficient, effective, cross-protective and particular development of vaccines these methods have some weak points such as restricted immunoinformatics tools to the linear epitopes, deficiency of B-cell epitope servers, deficiency of conformational epitope servers, and restrictions of the servers predictor of overlapping epitopes (12, 18, 23, 24).

So obtaining the useful results from immunoinformatics methods requires epitope database updating and should be coupled with experimental techniques.

In recent years, numerous studies have been conducted to introduce the effective peptide vaccines against infectious diseases. In this regard, Nazarian *et al.* introduced chimeric multi-subunit vaccine based on the B- and T-cell epitopes of virulence factors of enterotoxigenic *Escherichia coli* (25). In a similar study Hasan and his colleagues introduced novel peptide for corss protection against *Saint Louis encephalitis* virus infection (26). In another study Farhadi *et al.* designed a novel peptide that was composed of discontinuous (conformational) B cell and linear CD4+ T cell epitopes of the Outer membrane proteins (Omps) of Klebsiella pneumoniae to protect against this pathogenic bacterium (27). In the same context, Badawi *et al.* introduced a novel universal multi-epitope peptide vaccine in the whole spike glycoprotein of Middle East Respiratory Syndrome Coronavirus (MERS COV) (28). Due to the high efficiency and specificity of epitope-based vaccines for protection against infectious diseases, numerous attempts have been made to introduce epitope map of HCV virulence factors and effective epitope-based vaccines for protection against HCV infection. Huang *et al.* introduced a HCV dataset consisting of 774 linear B-cell epitopes and 774 non B-cell epitopes and revealed that PDREMVLYQE could be a conserved promising vaccine candidate for protection against HCV infection. But this peptide had no high antigenicity score in the present study (29). In a similar study, Ikram *et al.* confirmed that many epitopes from E2 protein are conserved in HCV 3a genotypes and HCV gpE2 is an ideal target for HCV vaccine design (30). In another study more probable and conserved T- and B-cell epitopes of E1 protein from HCV isolated in Pakistan were introduced as potential B- and T-cell epitopes that can raise the desired immune response against HCV (31). In this regard Gededzha *et al.* who predicted antigenic T-cell epitopes that bind HLA class II and HLA class I also showed that the highest number of HLA class I and HLA class II binding epitopes were predicted within the NS3 (63%) and NS3 (30%), respectively (32). In this study, we became eager to

design a novel chimeric peptide that can stimulate humoral and cellular immune responses to different serotypes of HCV. For this, more studied surface proteins of HCV were selected for determination of the conserved area of mentioned protein and construction of the chimeric peptide. The results showed that some areas of E1 and E2 proteins are conserved between different serotypes of HCV. More probable epitopes from mentioned proteins that were located in the conserved area were selected for construction of the chimeric peptide. So this peptide could be a good candidate for cross-protection against different serotypes of HCV.

Conclusion

The aim of this study was to determine B- and T-cell conserved epitopes of E1 and E2 proteins from HCV and to construct a chimeric peptide as a novel epitope-based vaccine for cross-protection against HCV. The results of this study showed that the chimeric peptide had high antigenicity score and stability. In conclusion epitope-based vaccine that is designed by immunoinformatics methods can be considered as a novel and effective platform of vaccines for cross-protection against HCV infection.

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Authors' contributions

M Nosrati participated in study designing, data collection, interpretation and manuscript writing. H Mohabatkar, data analysis and manuscript writing. M Behbahani, data interpretation and final approval of the manuscript.

Conflict of interest

The authors have no competing interests.

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