Original Article

Development of Polyepitopic Immunogenic Contrast Against Hepatitis C Virus 1a-6a Genotype by in Silico Approach

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Abstract

Background: Hepatitis C is a viral disease associated with chronic hepatitis and hepatocellular carcinoma. Hepatitis C virus (HCV) plays a critical role in the pathogenesis of this disease. Nonstructural proteins including NS3, NS4A, and NS5A are important in viral replication and translation. Since recent therapies are not appropriate for anti-HCV activity in humans, the main objective of this study is the use of immunoinformatic approaches for designing a novel multiepitope peptide with antigenic properties and examining it as a vaccine against (1a-6a) genotypes of the virus. These types of studies can be helpful for the development of new vaccine strategies against hepatitis C disease. **Methods:** The conserved position of nonstructural proteins (NS3/NS4a and NS5A) of HCV genotypes was used for vaccine design. Linear and conformational epitopes of B cell, MHC-I, MHC-II binding epitopes, and interferon-gamma inducing epitopes were determined in the construction of the vaccine. Molecular dynamics (MD) simulation and protein docking multiepitope peptides with toll-like receptor (TLR) 3 and TLR8 were analyzed. **Results:** MD simulation revealed a stable structure of candidate vaccines. Hence, docking results showed multiepitope peptides interaction with TLR3 and TLR8 and epitopes related to NS3 protein have the most interaction. These analyses suggest that designed vaccines can induce humoral and cellular immune responses against HCV. **Conclusions:** These analyses suggest that designed vaccines can induce humoral and cellular immune responses against HCV. However, experimental tests are required to evaluate the safety and immunogenicity profile of designed multiepitope vaccines.

Keywords: Epitope, hepatitis C virus, immunoinformatics, protein–protein docking, vaccine

Introduction

Hepatitis C virus (HCV) causes approximately 70% non-A, non-B hepatitis around the world.[1] This virus causes chronic hepatitis and hepatocellular carcinoma.[2] The 1a and 3a genotypes are the most frequent HCV genotypes in Iran.[3] Vaccines are currently available for hepatitis A and hepatitis B, but a vaccine for hepatitis C is still under investigation.[4] Since recent therapies are not appropriate for HCV infection treatment and clearance, designing a vaccine with high immunogenicity and necessary immune responses can be helpful for protection against infection and prevention of this disease. To design an effective vaccine against HCV, information about the HCV structure and replication components is needed. HCV has an RNA genome that encodes a polyprotein with 3000 amino acids. This polyprotein is cleaved by viral protease and creates structural (E1, E2, and C) and nonstructural (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) proteins.[1] Nonstructural proteins have important roles in the process of replication and maturation of HCV.[5] In the acute phase of HCV infection, specific T cell responses will be produced against nonstructural proteins[6] and also, several studies show that important regions of nonstructural proteins provide an affective T cell immunity response.[7,8]

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NS3 protein has serine protease and helicase activity. This helicase protein is essential for virus replication and translation. This protein also inhibits adenosine triphosphate-dependent protein kinases phosphorylation.[9] NS3 targets TRIF and MAVS proteins of the host immune system and functions as signaling adapters for toll-like receptor (TLR) 3 and mitochondrial antiviral signaling protein, respectively, and affects the host immune system.[10] Several studies have shown that T-cell immune response against NS3 is often associated with the resolution of the virus.[11,12] The NS4 protein is the NS3 cofactor for 4B/5A cutting.[13] The transportation's role from ER to Golgi is played by the NS4A/B protein.[14] Moreover, this protein induces apoptosis through the mitochondrial pathway by reduction of mitochondrial membrane potential, releasing cytochrome c and activating caspase-3, so this protein has a main role in cell hepatic death.[15] NS5A protein modulates cell cycle and immunoregulatory genes[2] and is critical in viral persistence and pathogenesis. This nonstructural protein of the virus interacts with a large number of hosts and viral proteins during replication and assembly.[16,17] NS5A has also an anti-apoptotic activity in mammalian cells. Signal transducers and activators of transcription (STATs) are cytoplasmic transcription factors that are activated in response to cytokines and growth factors. It plays an important role in the proliferation and prevention of apoptosis in the cell. JAK protein activates STAT by phosphorylation. NS5A can induce STAT phosphorylation by JAK and activate this factor.[2] In this study for designing an effective antigen-based vaccine, concerning the role of NS3/NS4A and NS5A non-structural proteins in the life cycle of HCV, these proteins were selected.

The CD8+ cytotoxic T lymphocytes and CD4+ T-helper cells are necessary for the control of HCV infection and are considered as the main effector cells in eliminating HCV-infected cells.[18] HCV-specific CD4+ T cells are involved in the initiation and maintenance of adaptive immunity. In addition, HCV-specific CD8+ T cells play a critical role in cytokine production, activation of antigen-presenting cells, B cell maturation, and regulatory functions.[19] There is a strong association between HLA-DRB1*0101, DRB1*1101, DQB1*0301 alleles, and viral infections. HLA-A*03, B*27, DRB1*0101, DRB1*0401, and DRB1*1501 alleles play a significant role in the acute phase of HCV removal.[20] HLA-DQB1*0301 alleles involved in the removal of viral infection are shown in colored-skin people. colored-skin people suppress viral infection by HLA-DRB1*0101 and HLA-DRB1*0101-DQB1 * 0501 alleles.[21] IgM and IgG (G1, G2, G3, and G4) antibodies are activated in response to HCV infection. IgG antibody produced in response to NS3, NS4, and NS5 proteins.[22,23]

Most vaccines can reduce viral infections through antibody responses and neutralize viral particles to minimize the proliferation of viruses.[24,25] One of the major problems in the development of an effective vaccine against HCV is the high genetic variability of HCV.[26] To overcome this problem, the computational vaccinology approaches are a successful option to design a multiepitope vaccine with high conservity against HCV.[27] In this study, the computational vaccinology approaches have been selected for designing new multiepitope peptide vaccines against HCV. Our vaccines contain several B-cell, T-cell, and interferon-gamma inducing epitopes of NS3/NS4A and NS5A proteins for (a) different genotypes of HCV.

Methods

The overall procedures used for vaccine design were categorized into six main parts: (1) conserved motifs selection in NS3 and NS4A and NS5A protein (1a-6a) genotypes of HCV, (2) evaluation of physical, chemical, and antigenicity properties of fusion peptide, (3) prediction of B-cell and MHC binding epitopes and interferon-gamma (IFNγ) inducing epitope, (4) selection of overlapped motifs detected by MHCI, MHCII, B cell, and IFNγ and linking them by GGGS linker and designing of other multiepitope vaccines, (5) three-dimensional (3D) structure prediction of constructed vaccines, and (6) molecular dynamics (MD) simulation and molecular docking analysis of constructed vaccines with immune receptors TLR3 and TLR8. An overview of the methods used for vaccine design is shown in Figure 1.

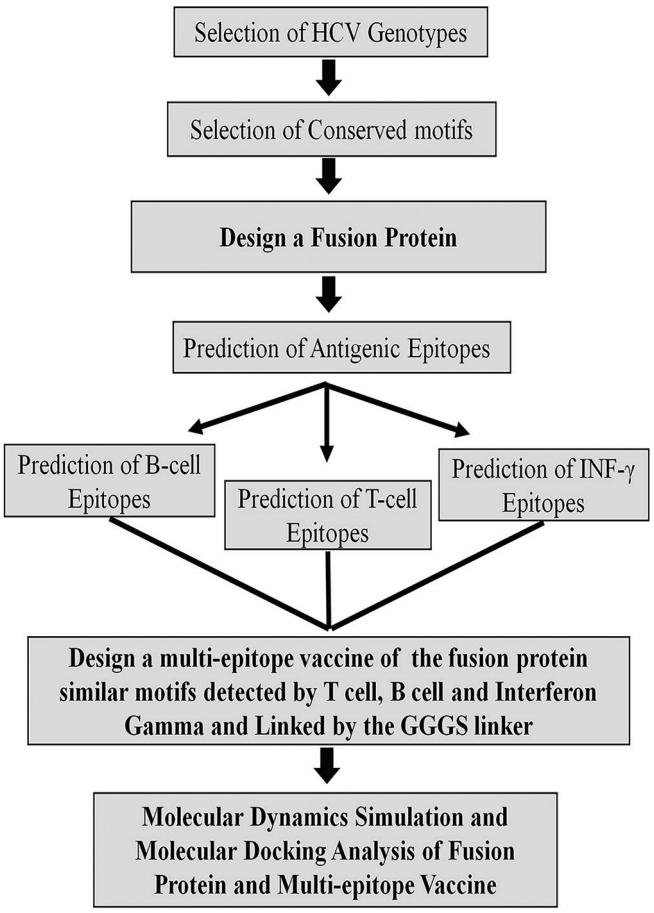

Figure 1: A summary of the methodology adopted for in silico design of the peptide vaccine in the study

Data collection

In this study, 1a, 2a, 3a, 4a, 5a, and 6a genotypes of HCV were selected as a model for antigenic epitope prediction. At least three clones of the NS3, NS4A, and NS5A protein sequences from the same patient were collected from the UniProt protein database (http://www.uniprot.org).[28] To design fusion protein, the sequences are submitted to the ClustalW server (http://www. genome. jp/tools/clustalw).[29] First, the sequences of collected clones from each genotype were aligned to obtain the consensus sequence. Finally, the obtained consensus sequences from all genotypes were aligned to conserve the sequence among all genotypes. Comparative analysis with human proteins was performed for selecting nonhuman homologs motifs by BLASTp.[30] Physical and chemical properties of designed vaccines, including the number of amino acids, molecular weight, aliphatic index, molecular weight, isoelectric point, and grand average of hydropathicity (GRAVY) were analyzed by ProtParam (http://web.expasy.org/protparam) of the ExPASy Bioinformatics Resource Portal.[31] Secondary and tertiary structures were predicted by (I-TASSER https://zhanglab.ccmb.med.umich.edu/I-TASSER/).[32]

Antigenicity identification

Vaxijen v2.0 is used for prediction antigenicity properties. This server predicts antigenicity based on the target organism such as bacterial, viral, and tumor antigens and determines antigenic probability based on the physicochemical properties.[33]

Prediction of B-cell epitopes

To predict B cell linear epitopes, the primary sequence of the peptide was submitted to BCpred (http://ailab.ist.psu.edu/bcpred/). This server predicts B cell linear epitopes by subsequences kernel and a support vector machine (SVM) with accuracy and specificity of 74.57 and 75%, respectively.[34] ABCpred (http://crdd.osdd.net/raghava//abcpred/) uses an artificial neural network which is based on applying fixed-length patterns with an accuracy of 65.93%, and a threshold of 0.51.[35] Conformational and discontinuous B cell epitopes were predicted by the ElliPro server (www.tools.immuneepitope.org/tools/ElliPro).[36]

Prediction of T-cell epitopes

Prediction of epitopes that interact with MHC and T-cells was determined by different servers including (a) EpiJen (http://www. ddg-pharmfac. net/epijen/EpiJen/EpiJen. htm) that predicts MHC binding epitopes based on quantitative matrices, derived by the additive method, and applied successively to select epitopes,[37] (b) MHCpred (http://www.ddg-harmfac.net/mhcpred/MHCPred/) with accuracy about 90% that predicts MHC binding motifs,[38] (c) IEDB (https://www.iedb.org/) that contains MHC binding data, antibody, and T cell epitopes for humans, nonhuman primates, rodents, and other animal species,[39] (d) Rankpep (http://imed.med.ucm.es/Tools/rankpep.html) uses the position-specific scoring matrices (PSSMs) for prediction of MHC-II binding epitopes.[40]

Prediction of interferon-gamma epitopes

In this study, IFN-γ inducing epitopes were predicted using the IFN epitope server (http://crdd.osdd.net/raghava/ifnepitope). This server generates all possible overlapping peptides form of antigen, based on their SVM score. The module identifies the best antigenic regions or IFN epitope in a query of antigen sequence that can induce IFN-γ.[41]

Another construction of the multiepitope vaccine

All of the selected epitopes were analyzed for overlap peptide epitopes that can bind to MHC-I, MHC-II, B-cell, and IFN-γ. Therefore, a GGGS flexible linker was added in each overlap peptide epitope sequence. The antigenicity property of the new multiepitope vaccine was calculated by Vaxijen v2.0. Finally, multiepitope vaccine and fusion protein structures were predicted by I-TASSER.

Molecular dynamics simulation and molecular docking analysis of the fusion protein and multiepitope vaccine candidate with toll-like receptor 3 and toll-like receptor 8 immune receptors

After modeling of the fusion protein and multiepitope vaccine, to stabilize the structure of the vaccine constructs, GROMACS 4.5.3 package, an MD simulation program, was used. MD simulation at 20 ns with 50,000 steps was run for equilibrated constructs. Root mean square deviation (RMSD) and radius of gyration (Rg) were obtained to predict dynamic behavior and structural changes of the studied two predicted protein vaccines and the results were generated in the form of graphs.

Molecular docking was performed between immune receptors (TLR-3 [PDB ID = 2A0Z] and TLR-8 [PDB ID = 3W3G]) and designed vaccines. For this protein–protein docking, the ClusPro server (https://cluspro.bu.edu/l) was applied.[42,43] This server is widely used for protein–protein docking. To analyze the schematic illustration of the interactions between docked proteins, the PDBsum online database (http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/) was utilized. PDBsum is a database that analyses protein secondary structure and protein-ligand and protein-DNA interactions.[44]

Results

In conversation analysis, regions with conversity >90% in NS3, NS4A, and NS5A proteins are selected for designing the fusion protein. The fusion protein comprised of 261 amino acid residues with 27,697.44 Da molecular weight. Based on ProtParam algorithms, the GRAVY, isoelectric pH, and its aliphatic index are − 0.034, 5.01, and 87.47, respectively. The two-dimensional structure of this peptide composes 26.1% helix, 13.4% sheet, 51% coil, 6.1% turn, and 3.4% 3–10 helix. The amino acid sequence of the fusion protein is shown NS3 (bold), NS4A (italic)., and. NS5A (line) (**APITAYAQQ TRGLDLYLVTRHADVIPARR RGDLHAPTGSGK STKVPAAYAAQGYKVLVLNPSVAATITYS TYGKFLADGGCSGGAYDIIICD ECILGIGTVLDQA**

ETAGVRLIKGGRHLIFCHSKKKCDELALGLN AVAYYRGLDVSVIPTTDALMTG FTGDFDSVIDCNP QDAVSRSQR RGRTGRGRTPGLPVCQDHLEF WEGVFTGLT*STWVLVGGVLAALAAYCLDEME ECV*GSQLPCEPE PDSSMPPLEGEPGDPDL).

Antigenicity assessment

VaxiJen prediction for the query sequence as an antigenic sequence with an overall prediction for the protective antigen was 0.5143.

Antigenic properties

B-cell epitopes

The B-cell linear epitopes were predicted with ABCpred, BCpred, and ElliPro servers. Table 1 shows some epitope sequences extracted by these three servers. For each epitope, sequence, start position, and score were indicated. Motifs with a score of more than 0.7 were listed.

Discontinuous B-cell epitopes

B-cell conformational and discontinuous epitopes of the fusion protein were predicted by the ElliPro server. This server identifies and rates the designed protein space epitopes based on 3D structure and reviews homology, so the tertiary structure of conformational epitopes with a score >0.5 was determined [Table 2 and Figure 2].

T-cell epitopes

T-cell-specific and MHC-I-related epitopes were predicted by EpiJen (with a score >7), MHCpred (with a $\log IC_{50}$ >7), and IEDB (with a Percentile_rank lower than 4) programs [Table 3]. Furthermore, MHC-II binding epitopes were predicted by Rankpep (with a score >7), MHCpred (with a $\log IC_{50}$ >7), and IEDB servers (with a Percentile rank <4) [Table 3].

Interferon-gamma inducing epitopes

IFN-γ inducing epitopes were predicted by the IFN epitope server. Table 4 shows epitopes sequences that extracted by this server, the score of them is >1.

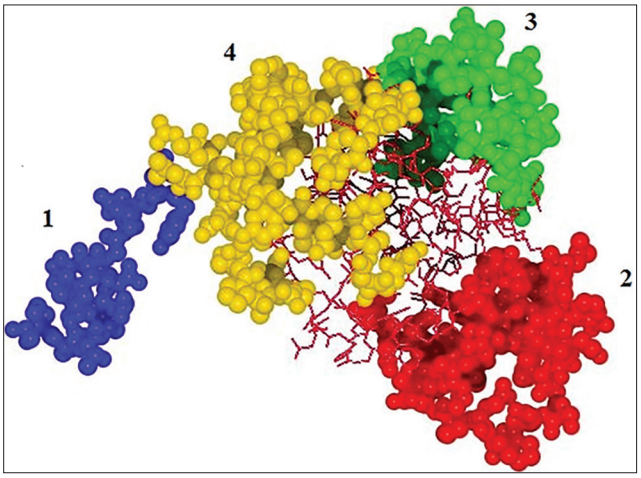

Figure 2: The three-dimensional structure of conformational B-cell epitopes predicted by the ElliPro server. The epitopes are spherical and colored

Table 1: B-cell linear motifs that were predicted for fusion protein presented in this study

Sequences	Start position	Score	Servers
PPLEGEPGDPDL	250	0.99	BCpred
GGCSGGAYDIII	79	0.99	BCpred
APTGSGKSTKVP	35	0.94	BCpred
IKGGRHLIFCHS	113	0.80	BCpred
LMTGFTGDFD	155	0.82	ABCpred
ITAYAQQTRG	3	0.79	ABCpred
YYRGLDVSVI	140	0.73	ABCpred
TRHADVIPAR	19	0.70	ABCpred
APITAYAQQTRGLDLYLVTRHADVIPAR	1	0.817	IEDB

High score: Good binder

Table 2: Position of B-cell conformational epitopes predicted by the ElliPro server

Number	Residues	Number of residues	Score
1	_:A1, _:P2, _:I3, _:T4, _:A5, _:Y6, _:A7, _:Q8, _:Q9, _:T10, _:R11, _:G12, _:L13, _:D14, _:L15, _:Y16	16	0.95
2	_:G208, _:L209, _:T210, _:S211, _:T212, _:W213, _:V214, _:L215, _:V216, _:G217, _:G218, _:V219, _:L220, _:A221, _:A222, _:L223, _:A224, _:A225, _:Y226, _:C227, _:L228, _:D229, _:E230, _:M231, _:E232, _:E233, _:C234, _:V235, _:G236, _:S237, _:Q238, _:L239, _:P240, _:C241, _:E242, _:P243, _:E244, _:P245, _:D246, _:S247, _:L252, _:E253, _:G254, _:E255, _:P256, _:G257, _:D258, _:P259, _:D260, _:L261	50	0.67
3	_:G115, _:G116, _:R117, _:H118, _:F121, _:E130, _:L131, _:A132, _:L133, _:G134, _:L135, _:N136, _:A137, _:V138, _:L144, _:D145, _:V146, _:S147, _:V148, _:I149, _:P150, _:T151, _:T152, _:D153, _:A154, _:L155, _:F159, _:F163, _:D164, _:S165, _:V166, _:I167, _:D168	33	0.62
4	_:L17, _:V18, _:T19, _:R20, _:H21, _:A22, _:D23, _:V24, _:I25, _:P26, _:A27, _:R28, _:R29, _:D32, _:L33, _:H34, _:A35, _:P36, _:T37, _:G38, _:S39, _:G40, _:K41, _:S42, _:T43, _:K44, _:V45, _:P46, _:A47, _:A48, _:Y49, _:A50, _:A51, _:Q52, _:G53, _:Y54, _:K55, _:P61, _:S62, _:V63, _:A64, _:A65, _:T66, _:I67, _:S82, _:G83, _:G84, _:A85, _:Y86, _:D87, _:I88, _:A108, _:G182, _:T184, _:G185, _:R186	56	0.60

Table 3: Prediction of MHC-I and MHC-II motifs of the fusion protein presented in this study

Sequence MHC-I	Allele	logIC_{50} (M)	Server
GIGTVLDQA	A0301	7.74	MHCpred
YLVTRHADV	A0301	7.63	MHCpred
PITAYAQQT	A0301	7.63	MHCpred
GLNAVAYYR	A0301	7.50	MHCpred, EpiJen
AAYAAQGYK	A0301	8.10	EpiJen, IEDB
DAVSRSQRR	A0301, B27	7.8	EpiJen
Sequence	**Allele**	**Percentile_rank**	**Server**
ITYSTYGK	A0301	3.4	IEDB
YRGLDVSVI	B27	0.79	IEDB
RRGRTGRGR	B27	0.43	IEDB, EpiJen
TRHADVIPAR	B27, A0301	0.45	IEDB, EpiJen
GRHLIFCHSKKKCD	B27, A0301	0.63	IEDB, EpiJen, MHCpred
Sequence MHC-II	**Allele**	**logIC_{50} (M)**	**Server**
GYKVLVLNP	DRB0101, DRB0401	8.89	MHCpred
PITAYAQQT	DRB0101	8.73	MHCpred
YLVTRHADV	DRB0101	8.63	MHCpred
STKVPAAYA	DRB0101	8.52	MHCpred
HADVIPARR	DRB0101	8.2	MHCpred
LVTRHADVI	DRB0101	8.1	MHCpred
WVLVGGVLA	DRB0401	7.5	MHCpred
GVLAALAAY	DRB0401	7.3	MHCpred
LVLNPSVAA	DRB0401	7.09	MHCpred
Sequence	**Allele**	**Score**	**Server**
VSRSQRRGR	DRB1*1101	10.807	Rankpep
YSTYGKFLA	DRB1*1101, DRB1*1501	9.16	Rankpep
DALMTGFTG	DRB1*1101	8.981	Rankpep
LEFWEGVFT	DRB1*1501	12.142	Rankpep
EGVFTGLTS	DRB1*1501	11.015	Rankpep
MTGFTGDFD	DRB1*1501	8.573	Rankpep
GLNAVAYYR	DRB1*1501	7.176	Rankpep
LVGGVLAAL	DQB1*0301	17.129	Rankpep
LDQAETAGV	DQB1*0301	13.895	Rankpep
FCHSKKKCD	DQB1*0301	11.29	Rankpep
FLADGGCSG	DQB1*0301	9.997	Rankpep
KVPAAYAAQ	DQB1*0301	8.923	Rankpep
Sequence	**Allele**	**Percentile rank**	**Server**
AVAYYRGLDVSVIPT	DRB1*04:01, DRB1*1501, DRB0101	0.4	IEDB
AQGYKVLVLNPSVAA	DRB1*04:01, DRB1*11:01, DQB1*0301,	0.98	IEDB
AAQGYKVLVLNPSVA	DRB1*04:01, DRB1*11:01, DRB1*1501	1.51	IEDB
YAAQGYKVLVLNPSV	DRB1*04:01, DRB1*11:01	1.71	IEDB
AYAAQGYKVLVLNPS	DRB1*04:01, DRB1*01:01, DRB1*11:01, DQB1*0301	1.81	IEDB
STWVLVGGVLAALAA	DRB1*01:01, DRB1*11:01	1.15	IEDB
QGYKVLVLNPSVAAT	DRB1*11:01	0.98	IEDB
VLAALAAYCLDEMEE	DQB1*05:01	0.40	IEDB
GVLAALAAYCLDEME	DQB1*05:01	0.59	IEDB

High score: Good binder, High Log IC_{50}: Good binder, Low percentile rank: Good binder. IC_{50}: Half maximal inhibitory concentration

Design of the new multiepitope vaccine

Overlap peptide motifs of the fusion protein detected by MHC-I, MHC-II, B cell, and IFN-γ linked through the GGGS linker. Secondary structure of the new protein composed of 18.2% helix, 9.4% sheet, 14.1% turn, and 58.2% coli. VaxiJen showed a query sequence as an antigen. The overall prediction for this protective antigen was 0.5973. Amino acid sequences of the multiepitope vaccine candidate with the GGGS linker is in the form (HAPTGSGKSTKVPAAGGGSGRHLIFCHSKK KCDGGGSLMTGFTGDFDGGG SITAYAQQTRGGGGS

YYRGLDVSVIGGGGSTRHADVIPAR GGGSYLV TRHADVGGGSGLNAVAYYRGGGSDAVSRSQRR GGGSYSTYGKFLAGGGSTGSGKSTKVPA AGGGSLVLNPSVAA).

Molecular dynamics simulation and molecular docking of the fusion protein and multiepitope vaccine candidate

The secondary and the tertiary-dimensional structures of the fusion protein and multiepitope vaccine candidates were predicted by I-TASSER [Figure 3a and b]. The energy minimization and MD simulation process of both models were made using the steepest descent algorithm of the GROMACS package. RMSD and Rg analysis are presented in Figure 3c and d. After 3 ns, the structure of the protein reached a certain distance from the reference structure and then was kept at that distance more or less, until it reached to the plateau on 54 ns and maintained its value.

Four molecular dockings using ClusPro 2.0 were performed between the fusion protein and the multiepitope vaccine candidate with immune receptors TLR3 and TLR8 to investigate the binding mechanism and interaction modes

Table 4: Prediction of interferon-gamma inducing epitopes for the fusion protein

Sequence	Start-end	Score
SGKSTKVPAA	38-48	1
VLNPSVAATI	57-67	1
GLDLYLVTRHA	11-22	1
TGSGKSTKVPAA	36-48	1
HAPTGSGKSTKVPAA	33-48	1

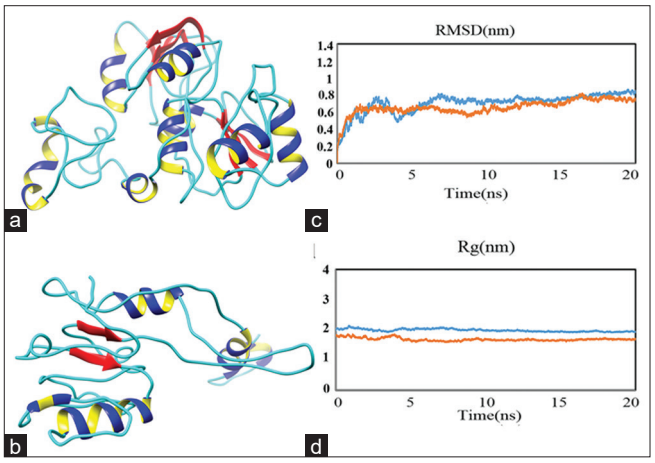

Figure 3: (a) Tertiary structures of the fusion protein and (b) multiepitope vaccine candidate predicted by the ITASSER server. (c) Root mean square deviation evolution and (d) radius of gyration. The fusion protein (blue) and multiepitope vaccine (orange)

Table 5: Interactions of the fusion protein and the multiepitope vaccine candidate epitopes with the desired receptors after docking

	Epitopes	Proteins	HLA alleles	Number of amino acids
Tol3 receptor-multiepitope vaccine candidate	HAPTGSGKSTKVPAA	NS3	DRB*0101, DQB1*0301	V (12), P (13), A (14), A (15)
	PITAYAQQT	NS3	A*0301, DRB*0101	Y (155), Q (157)
	AVAYYRGLDVSVIPT	NS3	B*27, DRB1*04:01, DRB1*1501, DRB*0101	D (71)
	TRHADVIPAR	NS3	B*27, A*0301	R (89)
	GLNAVAYYR	NS3	A*0301, DRB1*1501	N (109), V (111),A (112), Y (113), R (115)
	YSTYGKFLA	NS3	A*0301, DRB1*1101, DRB1*1501	Y (136)
	STKVPAAYAA	NS3	DRB*0101	A (156), A (157)
	LVLNPSVAA	NS3	DRB1*04:01, DRB1*11:01, DQB1*0301	L (162), V (168), A (169)
Tol8 - multiepitope vaccine candidate (A chain)	GLNAVAYYR	NS3	DRB1*1501, A*0301	N (109), A (110), V (111), Y (113)
	TGSGKSTKVPAA	NS3	DRB and 0101	T (152), P (155)
	LVLNPSVAA	NS3	DRB*0401 , DRB1*11:01, DQB1*0301	L (162), V (163), L (164), N (165), S (167), V (168)
B chain	GLNAVAYYR	NS3	DRB1*1501, A*0301, DRB1*04:01, DRB*0101	A (112), R (115)
Tol3 - fusion protein	PITAYAQQT	NS3	A*0301	T (4), A (5)
	TRHADVIPAR	NS3	B*27, A*0301	D (23),P (26), A (27)
	TGSGKSTKVPAA	NS3	DRB*0101	T (43), K (44), A (47)
	AAQGYKVLVLNPSVAA	NS3	DRB1*04:01, DRB1*11:01, DRB1*1501, DQB1*0301, DRB1*01:01	A (50), A (51), G (53), Y (54)
	ITYSTYGK	NS3	A*0301	I (66), T (67)
	LDQAETAGV	NS3	DQB1*0301	E (106)
	VSRSQRRGR	NS3	DRB1*1101	R (177), R (181)
	LEFWEGVFT	NS3	DRB1*1501	L (199), E (200)
Tol8 - fusion protein (A chain)	STWVLVGGVLAALAA	NS4A	DRB1*01:01, DRB1*11:01, DQB1*05:01	S (211), V (214), L (215), L (220), A (221), A (222)
	AVAYYRGLDVSVIPT	NS3	DRB1*04:01, DRB1*1501, DRB*0101	R (142), G (143), L (144), D (145)
B chain	VLAALAAYCLDEMEE	NS4A	DQB1*05:01	C (227), L (228), D (229), E (230), E (233)

of these two receptors. For each molecular docking result, among the 20 models built by this server, the best model with the most clusters and the lowest energy was selected [Figures 4a-d and 5a-f]. After docking, interactions of epitopes with the desired receptors were investigated [Table 5].

Discussion

In the current research, two multiepitope vaccines were designed. The base of these vaccines is related to conserved B-cell, T-cell, and IFN-γ epitopes of NS3/NS4A and NS5A. The vaccines can induce a huge level of the cellular and humoral immunity response against HCV infection. Hence, they can be studied as new vaccines for protection against HCV. Today, designing of an effective vaccine against HCV infection is an interesting field. Nosrati and *et al*. design a multiepitope vaccine against E1 and E2 proteins of HCV. The results showed that E1 protein had more epitopes than E2 protein.[45] Zhu *et al.* designed a novel adeno-associated virus (AAV) expression vector including the full-length NS3 or NS3/4 protein of HCV genotype 1b. This candidate could well stimulate the immune system but cannot be used for another genotypes.[46] Ikram *et al*. explored NS3/4A, NS5A, and NS5B protein for designing a multiepitope protein as a vaccine for HCV. The results of this study indicated that the designed polypeptide had potentially antigenic properties.[8]

Vaccine new programs against HCV can induce both humoral and cellular immune responses. However, due to the high diversity of the virus (existence of quasispecies), it can escape from the immune system.[47,48] Therefore, in designing an effective vaccine, selection of proteins and peptides with high antigenic properties is a better option to stimulate the immune response, since effective stimulation of the immune system will prevent and control this disease. An antigenic peptide should be contained epitopes for the B-cell receptor and also included MHC associated motifs that were detected by T lymphocyte cells receptor.[42] Immunoinformatics is an *in silico* new approach for the analysis and modeling of immunological data. One of the major applications of immunoinformatics is the design of epitope-based vaccines.[49,50] The multiepitope peptide vaccines are one of the vaccines against infections. These vaccines include B-and T-cell epitopes that induce an immune response.[51] Prevention and inhibition of HCV are the most important aims of the multiepitope T-cell vaccines.

The results of our research revealed that designed peptide vaccines provided effective cellular and humoral immunity responses to (a) serotype of HCV. B lymphocytes produce

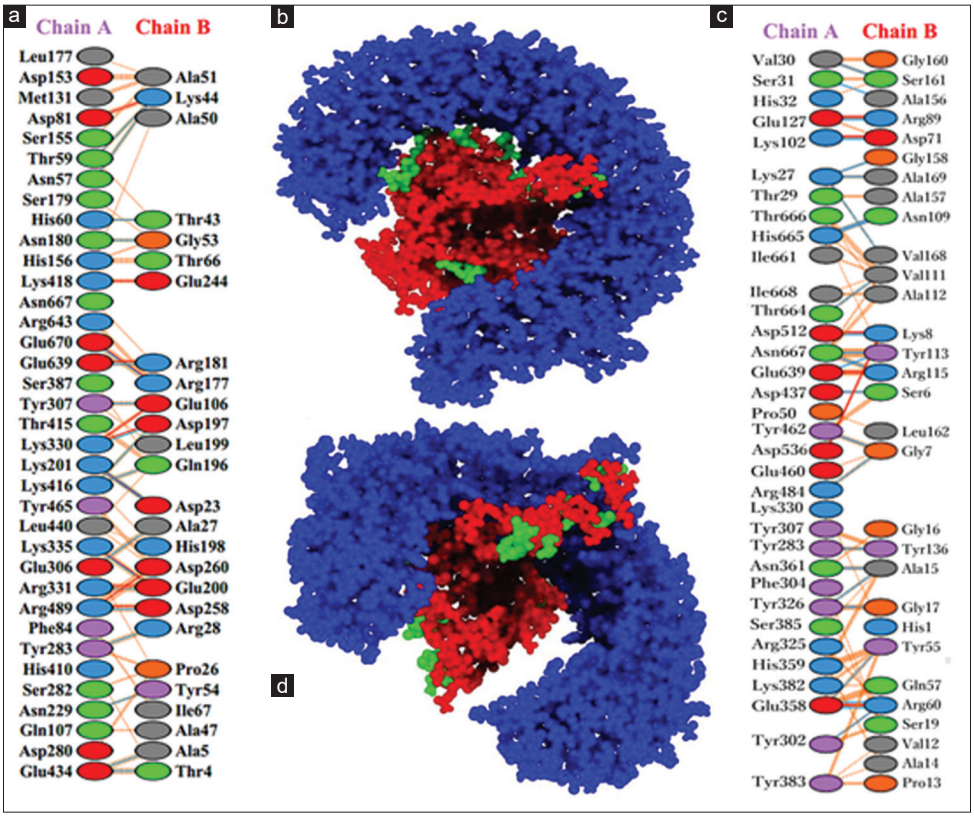

Figure 4: Docking of vaccines with TLR-3. (a) An illustration of interacting residues between docked fusion protein vaccine (chain B) and TLR3 (chain A). (b) The fusion protein vaccine construct-TLR3 docked complex: Figure obtained after molecular docking between fusion protein vaccine and TLR-3, the red color shows the fusion protein vaccine construct while blue color is representing TLR-3. (c) An illustration of interacting residues between docked multiepitope vaccine (chain B) and TLR3 (chain A). (d) Multiepitope vaccine construct-TLR3 docked complex: Figure obtained after molecular docking between multiepitope vaccine and TLR-3, the red color shows multiepitope vaccine construct, while blue color is representing TLR-3. Interacting residues are shown in both docks with green color. TLR: Toll-like receptor

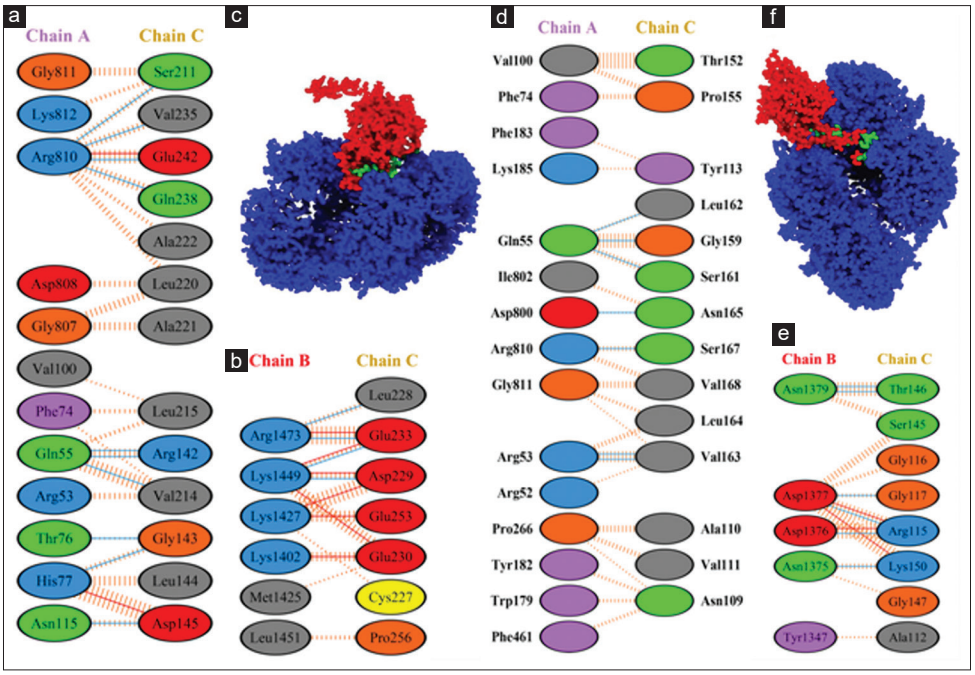

Figure 5: Docking of vaccines with TLR-8. (a and b) An illustration of interacting residues between docked fusion protein vaccine (chain C) and chain A and B related to TLR8, respectively. (c) The fusion protein vaccine construct-TLR8 docked complex: Figure obtained after molecular docking between fusion protein vaccine and TLR-8, the red color shows the fusion protein vaccine construct, while blue color is representing TLR-8. (d and e) An illustration of interacting residues between docked multiepitope vaccine (chain C) and chain A and B related to TLR8, respectively. (f) Multiepitope vaccine construct-TLR8 docked complex: Figure obtained after molecular docking between multiepitope vaccine and TLR-8, the red color shows the multiepitope vaccine construct, while blue color is representing TLR-8. Interacting residues are shown in both docks with green color. TLR: Toll-like receptor

specific secretion antibodies and have an important role in the immune memory and clearance of the pathogens. In the fusion protein, the linear epitope with a high score of 0.99 belongs to PPLEGEPGDPDL, and GGCSGGAYDIII was predicted by BCpred. These are located in NS5A and NS3 proteins respectively. In ElliPro, A1, _:P2, _:I3, _:T4, _:A5, _:Y6, _:A7, _:Q8, _:Q9, _:T10, _:R11, _:G12, _:L13, _:D14, _:L15, _:Y16 have high score (0.95) in discontinuous B-cell epitopes and located in NS3 protein.

The CD4+ T cell has an important role in innate and adaptive immune responses and CD8+ T cell involves in the destruction of virally infected cells,[52] both CD4+ and CD8+ T cell-related epitopes are important in vaccine efficacy. In this study, MHCpred, EpiJen, Rankpep, and IEDB servers were used to determine MHC-I and MHC-II associated peptide epitopes. According to the results of the prediction by used servers [Table 3], for the HLA-A0*301 allele (GIGTVLDQA (logIC_{50} [M] = 7.74) and TRHADVIPAR [Percentile_rank = 0.45]) epitopes and the HLA-B*27 allele (RRGRTGRGR [Percentile_rank = 0.43]) epitope that belong to the NS3 protein have potential to be processed and presented by MHC I molecules. HLA-DRB0*101 and HLA-DRB0*401 Alleles, (GYKVLVLNP (logIC_{50} [M] = 8.89)) epitope, the HLA-DRB1*1101 allele (VSRSQRRGR [score = 10.807]) epitope and the HLA-DRB1*04:01, HLA-DRB1*1501 and HLA-DRB0*101 alleles (AVAYYRGLDVSVIPT [percentile rank = 0.4]) epitope that be able to present with MHC II molecules, also belonged to the NS3 protein.

We performed docking of TLR-3 and TLR-8 with designed proteins for analyzing the immune response of TLR-3 and TLR-8 against predicted vaccines. TLR3 reduces viral load in HCV-positive patients,[53] and TLR8 plays an important role in inducing antiviral immune responses against HCV.[54] These results showed that the epitopes related to NS3 protein have the most interaction with TLR-3 and TLR-8.

In the designed multiepitope vaccine, overlap peptide epitopes of B cell, T cell, and IFN-γ epitopes have been selected to design the vaccine. The epitopes of overlapped B cell, T cell, and IFN-γ can promote both B and T cell responses and also induce produced pro-inflammatory cytokines with antiviral activity by IFN-γ.[55] The multiepitope vaccine has higher antigenicity properties than the fusion protein vaccine and also makes strong binding to TLR-3 and TLR-8 receptors.

Conclusions

We determined B-cell, T-cell, and IFN-γ epitopes of NS3/NS4A and NS5A from (a) genotype HCV and design immunogenic multiepitope vaccines as epitope-based vaccines. These vaccines can be used for protecting against HCV infection. However, *in vitro* and *in vivo* immunological tests are needed to validate the efficiency of designed vaccines.

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Conflicts of interest

There are no conflicts of interest.

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