Sdü Sağlık Bilimleri Dergisi / Cilt 11 Sayı 2 / 2020

Design and Analysis Of Recombinant Vaccine Against *Clostridium Perfringens* Type A and Type E

Clostridium Perfringens Tip A ve Tip E'ye Karşı Rekombinant Aşı Tasarımı ve Analizi

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Abstract

Objective: *Clostridium perfringens (C. perfringens)* is a spore-forming, anaerobic, gram positive, bacteria that found in environment, soils, foods, and in intestinal traces of humans or animals. However, vaccines being developed for use in animals have the potential to be developed for use in humans. This study was aimed to design and analysis of multi epitope vaccine against *C. perfringens* type A and E.

Material-Method: The B cell epitopes were predicted by IEDB (https://www.iedb.org/) and MCH Class II epitopes were predicted by Vaxign 2 Beta (http://www.violinet.org/vaxign/) and Propred-I 2003 (http://crdd.osdd.net/raghava/propred1/) web server.

Results: Physicochemical study of vaccine showed that the designed vaccine is 58.33 kDa. The half-life of candidate vaccine was found to be greater than 100 hours in mammalian cells, greater than 20 hours in yeast, and greater than 10 hours in *Escherichia coli*. The instability index of vaccine was 28.41 (<40), the aliphatic index was found to be 47.51, and the vaccine is soluble in water and is considered stable. The grand average of hydropathicity of candidate vaccine is -1.283, consequently, the vaccine is a hydrophilic protein and easily soluble in water. The result obtained from of ToxinPred and AllerTop revealed that the protein don't have toxic and allergenic effect on human and animal cells. PepCalc and ProtParam analysis showed that the vaccine doesn't have transmembrane helix in its structure, so no expression difficulties are expected in the development of the protein from recombinant DNA technology methods. The result obtained from docking analysis proved that the vaccine has maximum affinity to HLA-DRB1*0101 with the score of -660.73.

Conclusions: The result of this study showed that the candidate vaccine can be stimulate HLA-DRB1*0101 and other MCH Class II alleles.

Keywords: B Cell, *Clostridium Perfringens*, HLA-DRB10101, T-Cell, Vaccine.

Özet

Amaç: *Clostridium perfringens (C. perfringens)*, çevrede, topraklarda, gıdalarda ve insanların veya hayvanların bağırsaklarında bulunabilen ve spor oluşturan, anaerobik ve gram pozitif bakteridir. Hayvanlarda kullanmak için geliştirilen aşılar insanlar tarafından da kullanma potansiyeline sahip olabilir. Bu çalışmada, *C. perfringens* tip A ve E'ye karşı çoklu epitop aşısının tasarlanması ve analizi amaçlanmıştır.

Materyal-Metot: B hücresi epitopları IEDB (https://www.iedb.org/) ve MCH Class II epitopları ise Vaxign 2 Beta (http://www.violinet.org/vaxign/) ve Propred-I 2003 (http://crdd.osdd.net/raghava/propred1/) programın kullanarak tasarlanmıştır.

Bulgular: Elde edilen fizikokimyasal sonuçlara göre tasarlanan aşı 58,33 kDa ağırlığına sahip olmaktadır. Aday aşının yarılanma ömrü memeli hücrelerinde 100 saatin üzerinde, mayada 20 saatin üzerinde ve *Escherichia coli*'de 10 saatin üzerinde olduğu bulunmuştur. Aşının instabilite indeksi 28,41 (<40), alifatik indeks 47,51 olarak bulunmuştur, aşı suda çözünür ve stabil yapıya sahiptir. Aday aşının genel hidropatisitesi -1,283'tür, sonuç olarak aşı hidrofilik bir proteindir ve suda kolayca çözünür. ToxinPred ve AllerTop analizlerinden elde edilen sonuçlara göre tasarlanan aşı insan ve hayvan hücrelerinde toksik ve alerjenik etkiye sahip değildir. PepCalc ve ProtParam analizi sonucunda aşının yapısında transmembran sarmalının bulunmadığı ortaya çıkmıştır, bu nedenle aşının rekombinant DNA teknoloji yöntemlerinden geliştirilmesinde herhangi bir ekspresyon zorluğu beklenmemektedir. docking analizinden elde edilen sonuçlara göre aşı HLA-DRB1 * 0101'e -660,73 skoru ile maksimum afiniteye sahip olduğunu kanıtlamıştır.

Sonuç: Bu çalışmanın sonucu aday aşının HLA-DRB1*0101 ve diğer MCHII alellerini stimüle edebileceğini göstermiştir.

Anahtar kelimeler: Aşı, B Hücre, *Clostridium Perfringens*, HLA-DRB1 0101, T-Hücre.

DOI: 10.22312/sdusbed.680719
Müracaat tarihi / Received date: 28.01.2020
Kabul tarihi / Accepted date: 21.05.2020
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Introduction

Clostridium perfringens (C. perfringens, other name is *Bacillus welchii*) is a spore-forming, anaerobic, gram positive, bacteria that found in environment, soils, foods, and in intestinal traces of humans or animals. The bacteria is ranked as significant pathogens for humans or livestock (1, 2). In the United States of America the most important reasons of food poisoning is *C. perfringens*. The infections of this bacteria cause's tissue necrosis in human and animals. *C. perfringens* produces a toxin which name is *C. perfringens* toxin. It has role in gas gangrene and myonecrosis in infected tissues (3). *C. perfringens* is a most important cause of histotoxic infection in humans and animals. The disease initiating in the human and animal intestines for instance enterotoxemia and enteritis. There are five type of C. perfringens that produce four different toxins: alpha (α), beta (β), epsilon (ε) and iota (I) (4, 5)

The alpha toxin is essential virulence factor for *C. perfringens* which is lethal and has enzymatic activity (6). Alpha toxin hydrolyzes the phospholipids of cell membrane and it has important role in gas gangrene. The structure of Alpha toxin showed that the toxin is consisting of N-domain and C-domain. The toxin uses from C-domain for binding to host membrane lipid bilayer (7). Beside human *C. perfringens* causes yellow lamb disease in sheep. Beta toxin is produced by types B and type C of *C. perfringens* which has important role in central nervous signs and mucosal necrosis in domestic animals. The type B of bacteria and toxin causes disease in animals and human (necrosis effect in human cells). *C. perfringens* encodes a cpb gene (*C. perfringens* beta toxin) that translate a 336 amino acids peptide, and 27-amino acid of this protein is as signal peptide which removed through secretion(8). The disease of types B and type C initiates in intestine of host (The bacteria produces the toxin in the intestine), so the toxin into circulation and leads to death of infected host(9). Epsilon toxin is a pore forming toxin that causes oedema. It is produced by type B and type D of bacteria and causes fatal enterotoxemia. Iota toxin is an ADP (Adenosine diphosphate ribose) ribosyltransferase that binds to cell surface protein and subsequently translocates iota toxin into the cytosol of host cell (10). This results in inhibition of cell functions by actin filament depolymerization. The role of iota toxin as a virulence factor is unknown. In one report, iota toxin showed positive effects on adherence and colonization of *C. perfringens* type E by altering the enterocyte morphology and strongly inhibit intra-specific growth of other strains (11). The alpha-toxin is important role in human and animal disease. There are no licensed vaccines for use in humans and animals which protect against either gas gangrene. However, vaccines being developed for use in animals have the potential to be developed for use in humans.

This study was aimed to design and analysis of multi epitope vaccine against *C. perfringens* type A and E.

Material and Methods

In this study we selected five protein from Type A and Type E of *C. perfringens* and the vaccine was designed by sequences of that proteins.

The protein sequence of Chain A, *C. perfringens* Alpha toxin (Genbank: 1KHO_A), NlpC/P60 family protein (GenBank: KXA13795.1), cell wall anchor domain protein (GenBank: PZT51328.1), peptide chain release factor 2, and (GenBank: RQN18066.1), peptide chain release factor 3 (GenBank: RUR35220.1) was taken from NCBI.

Antigenic effect of these proteins were checked by VaxiJen server. The vaxiJen score proteins were above the threshold 0.4. Allergicity and Ctotoxicity of the ptoteins were analyzed via AllerTop and ToxinPred webserver. We removed the part of proteins that were toxic for humans. The result obtained from AllerTop showed that the proteins don't have allergic potential for eukaryotic cells. Afterward, the epitopes of B cells were extracted by Immune Epitope Database analyzing software. IEDB (https://www.iedb.org/) utilizes an artificial neuron network for ensuring B-cell epitopes. In our research we selected epitopes that showed higher than 0.35 score. For prediction of T cell epitope (MCH Class II) we used from Vaxign 2 Beta (http://www.violinet.org/vaxign/) and Propred-I 2003 (http://crdd.osdd.net/raghava/propred1/) softwares. In these software's the epitopes were appraised for their binding affinity to different MCH Class II (dominant HLA II alleles) with P value of <0.05 were regarded as significant (12).

Antigenic effect of selected epitopes were checked using VaxiJen webserver (13). B cell and T cell epitopes were linked together by Lysine-Lysine (KK) amino acids linker and constructed a peptide chains. Afterward, the allergenicity, toxicity and antigenicity of multy-epitope vaccine were analyzed using AllerTop, ToxınPred, and VaxiJen softwares (14, 15).

This vaccine is designed for using in human and animals, and will be produce by recombinant DNA technology methods in future, for this reason we should check the solubility of the vaccine in human and *E.coli*. For this reason we used ProtParam web software to calculate of tendency of candidate vaccine to be soluble in human and *E.coli* as overexpression bacterial host cell (16). Aliphatic index, Molecular weight, Half-life, isoelectric index, and stability of designed vaccine investigated using PapCalc and ParatParam (17). Calculation of potential transmembrane helices was analyzed by Parabi software. So, 3D structure of our vaccine was was taken from Swiss-Model server. 3Drefine program was used to for refining of model of candidate vaccine. GDT-HA score, 3D refine score, GTD-TS, MolProbity, and RMSD score were taken from 3D refine program. The best model (high score) was chosen to Ramachandran analysis that was done Procheck online software (18). The server assigns to abnormal amino acids position which are in protein structure. The binding affinity of vaccine to MHC (HLA-DRB1*0101) investigated via HEX protein protein docking web server. Firstly, 3D structure of MCH Class II (HLA-DRB1*0101 that is one of predominant allele was taken from protein data bank (http://www.rcsb.org/structure/5V4N). Hex protein protein docking software calculates the energy which is needed to protein ligand docking (http://hex.loria .fr/manual800/hex_manual.pdf). In this study to ensure the validity of the work we used the 3D structure of human serum albumin as

negative control and calculated the affinity of the protein to HLA-DRB1*0101(19). The vaccine is most effective if it is recognized by other MHCII, for this reason we used from EPIDOCK for checkıng of the affinity of candıdate vaccıne to others MCH Class II types.

Results

B cell epitopes of *C. perfringens* vaccine were predicted by IEDB (Epitopes higher than 0.35 threshold) and for prediction of T cell epitopes we used from Vaxign (P-values <0.05 were choosen). The antigenic effect of selected epitopes of candidate vaccine was analyzed using VaxiJen, the result from VaxiJen showed that atigenic score of our vaccine is 1.023, the score revealed that the vaccine can be stimulate immune system (Figure 1).

The allergenecity and toxicity of vaccine evaluated by ToxinPred and Allertop web server. The obtained from software showed that the vaccine don't have toxic and allergic effect on our cells (Figure 2 and 3).

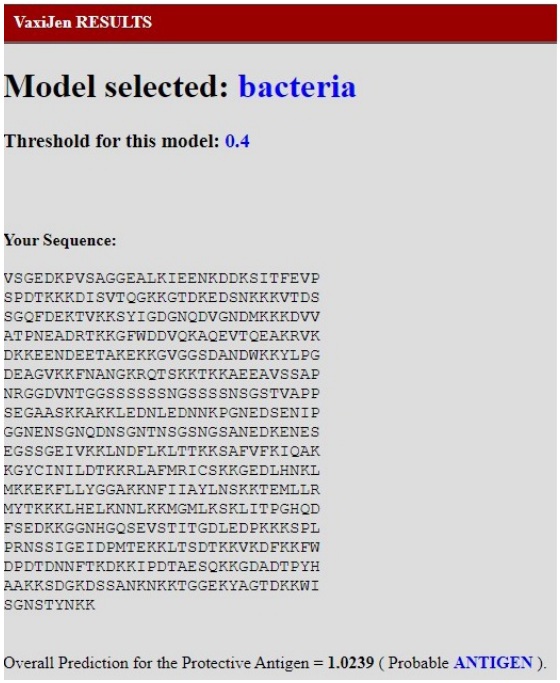

Figure 1. VaxiJen analysis of *Clostridium perfringens* vaccine

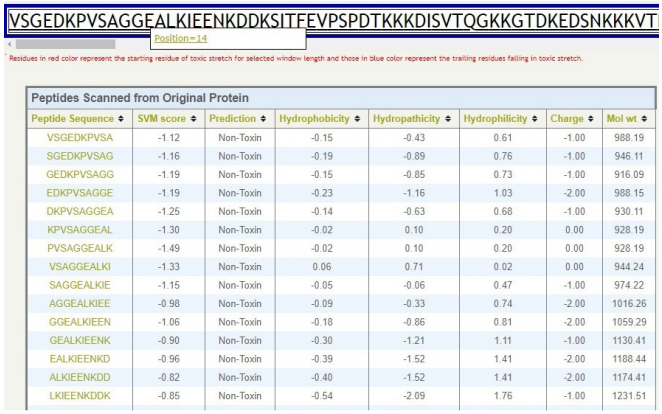

Figure 2. Prediction of toxicity of *clostridium perfringens* vaccine

Physicochemical study of designed vaccine showed that the molecular weight of vaccine is 58.33 kDa with 534 amino acids. The half-life time of *C. perfringens* vaccine was found to be greater than 100 hours in mammalian cells, greater than 20 hours hours in yeast and greater than 10 hours in *Escherichia coli*. The instability index (II) of candidate vaccine calculated as 28.41 (which is less than 40), the data obtain from ProtParam showed that the vaccine was stable. Beside that ProtParam showed that the Aliphatic index of vaccine is 47.51, the result obtained from alphalitic index analysis revealed that the the vaccine is thermostable. The hydropathicity index of candidate vaccine was was -1.283, the score revealed that the vaccine is a hydrophilic protein and easily dissolve in water.

The result obtained from PepCalc alalysis showed that the vaccine don't have transmembrane helix and it can be clone and express in *E.coli*. The transmembrane helix of vaccine is 29.85% (Figure 4).

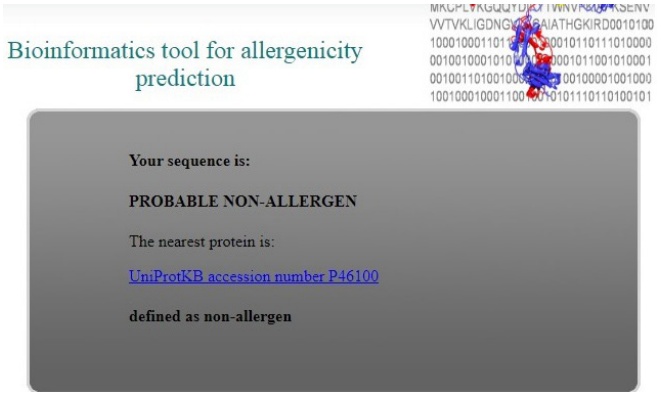

Figure 3. Prediction of allergenisity of *clostridium perfringens* vaccine

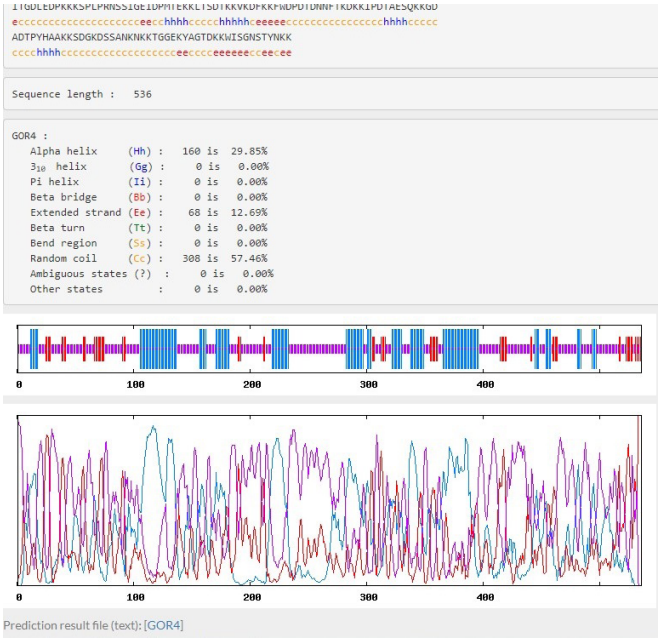

Figure 4. Secondary structure of *clostridium perfringens* vaccine

In this research we used from Swiss Model web server to drawing of 3D structure of multi epitope vaccine (Figure 5), then the 3D structure of vaccine was refined by 3D refine server. The result obtained from 3D refine analysis showed that the 3D refine score of vaccine was 2910, the GTD-TS (similarity score) score of vaccine was 1.000, the GDT-HA score (similarity score) was 1.000, the RMSD (deviation score) score was 0.212, the Molprobity score (physical realism score) was 2.383 and RWplus (potential energy) was -3726 (Figure 6).

Figure 5. 3D model of *clostridium perfringens* vaccine

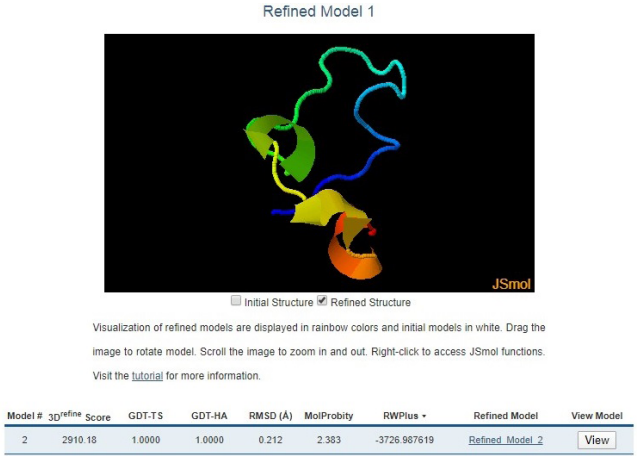

Figure 6. 3D refinement model of *clostridium perfringens* vaccine

Ramachandran plot showed that 86.7% of amino acids are in ideal regions, 13.3% in allowed regions and 0% of amino acids is in non-ideal regions (Figure 7). The Ramachandran plot proved the high quality structure of candidate vaccine.

HLA-DRB1*0101 one of predominant allele of MHCII, for this reason we investigated the affinity of vaccine to this MHC. Also, human serum albumin was selected as negative control and affinity of human serum albumin to HLA-DRB1*0101 was investigated via Hex. The result of docking analysis revealed that the candidate vaccine has maximum affinity to HLA-DRB1*0101 with the score of -660.73 whereas the affinity of negative control to HLA-DRB1was -65.30 (Figure 8). Beside that we used from another docking software to investigate the affinity of our designed vaccine to all of MHCII alleles. The result obtained EPIDOCK showed that our vaccine has high affinity to GEDKPVSAG epitope with the score of 0.305, EALKIEENK epitope with the score of 0.407, KDDKSITFE epitope with the score of 0.437, KSITFEVPS epitope with the score of 0.357, TFEVPSPDT epitope with the score of 0.363, and TKKKDISVT epitope with the score of 0.669 (Figure 9). The result of this study showed that the candidate vaccine can be stimulate HLA-DRB1*0101 and other types MHCII.

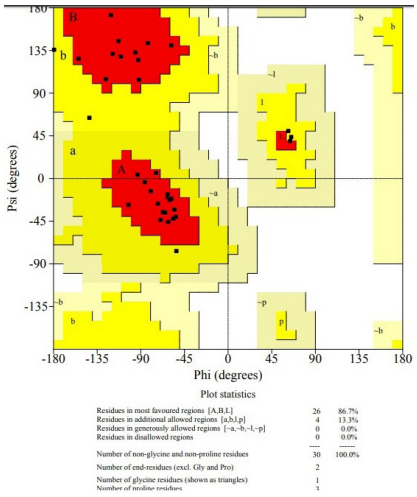

Figure 7. The ramachandran plot of *clostridium perfringens* vaccine

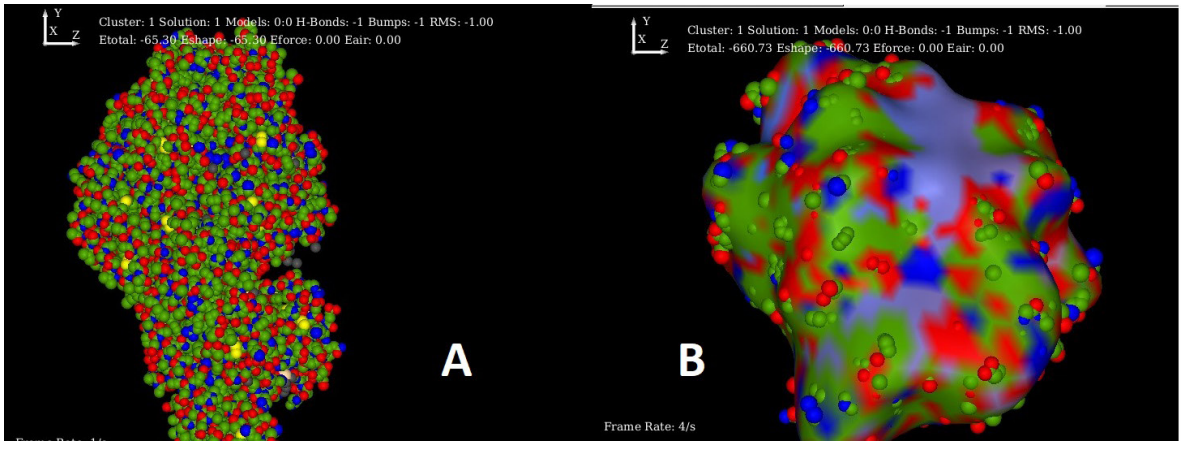

Figure 8. Molecular docking of HLA-DRB1*0101 with *clostridium perfringens* vaccine (A) and human serum albumin (B)

Name	Position	Sequence	DRB1*0101
0		Binders have score above or equal to the threshold given in this row.	0.3
seq_44449	3	GEDKPVSAG	0.305
seq_44449	13	EALKIEENK	0.407
seq_44449	21	KDDKSITFE	0.437
seq_44449	24	KSITFEVPS	0.357
seq_44449	27	TFEVPSPDT	0.363
seq_44449	35	TKKKDISVT	0.669
seq_44449	37	KKDISVTQG	0.466
seq_44449	43	TQGKKGTDK	0.459
seq_44449	54	SNKKKVTDS	0.476
seq_44449	55	NKKKVTDSS	0.471
seq_44449	58	KVTDSSGQF	0.371
seq_44449	63	SGQFDEKTV	0.699
seq_44449	66	FDEKTVKKS	1.053
seq_44449	69	KTVKKSYIG	0.494

Figure 9. Molecular docking of HLA-DRB1*0101 with c*lostridium perfringens* vaccine

Discussion

In this research we used the sequence of five protein sequence of *C. perfringens* type A and Type E for designing of multi epitope vaccine. The researcher were used bioinformatics software's and web servers to design and investigate the properties of the vaccine. The antigenic score of vaccine was 1.023, high antigenic score of different epitopes simply recognized by T-cells and B-cell, the peptides with antigenic properties are necessary to raise the immune responses. As suggested by the antigenicity analysis by VaxiJen, the vaccine candidate seemed to be effective against *C. perfringens*.

The significant evidences were established (secondary structure and physicochemical characters of designed vaccine) by Procheck, ProtParam and Parabi web servers. Physicochemical study of our designed vaccine revealed that the designed vaccine has a molecular weight of 58.33 kDa. Proteins that having <110 kDa molecular weight are suitable vaccine(20). The instability index of vaccine was 28.41 that is fewer than 40, consequently the vaccine is stable. The abundance of random coils in our candidate vaccine confirmed the higher stability of that protein (15, 21). The prediction of half-life of proteins is one of important challenge in vaccine design(22). The half-life time of *C. perfringens* vaccine was found to be greater than 100 hours in mammalian cells, greater than 20 hours in yeast and greater than 10 hours in Escherichia coli. The vaccine has high alphalitic index value, this score revealed that the vaccine is thermostable, consequently can be used in endemic and other area(23).

The 3D refines structure of candidate vaccine that was drawn by 3D refine server was acceptable.

Because the 3D refine score of vaccine was high (2910) and the GTD-TS score of vaccine was 1.000. Ramachandran analysis of vaccine revealed that greatest of amino acids are in favoured regions and 0% of residues are in outlier region, the result of Ramachandran plot proved that the quality of protein structure is satisfactory (16).The result of this study showed that our designed vaccine has the strongest affinity to HLA-DRB1*0101and other MHC alleles.

Conclusion

The result of this study proved that the vaccine has appropriate structure and docking analysis showed that the vaccine is stable in human and animal cell and can activate humoral and cellular immune responses against of *C. perfringens* type A and Type E. The vaccine can be cloned and purified in E.coli, which should be tested in model animals to prove its effectivity.

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