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In silico Analysis of Epitope-Based CadF Vaccine Design against *Campylobacter jejuni*

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Abstract

Objective: Vaccination is an important strategy for the eradication of infectious diseases. CadF protein of *Campylobacter jejuni* is one of the important factors in the pathogenesis of this bacterium. The purpose of this work was to perform a bioinformatics study to identify an epitope-based CadF vaccine, as a subunit vaccine. Full protein sequences of CadF were extracted from the NCBI and UniProt databases and subjected to *in silico* evaluations, including sequence analysis, allergenicity, antigenicity, epitope conservancy, and molecular docking assessments done by different servers.

Results: The results showed that CadF was a highly conserved protein belonging to the outer member proteins superfamily. Among the evaluated epitopes, LSDSLALRL was identified as an antigenic and non-allergenic peptide with a suitable structure for vaccine development. It was also able to stimulate both T and B cells. This 9-mer peptide was located in 136-144 segment of CadF protein and interacted with both HLA-A 0101 and HLA-DRB1 0101 alleles. Overall, the obtained theoretical results showed that CadF protein could be used for designing and evaluating a new effective vaccine against *C. jejuni*.

Introduction

Campylobacter jejuni (*C. jejuni*) is one of the significant pathogens belonging to the genus *Campylobacter*. The bacterium is a Gram-negative, curved, flagellated, and rod-shaped pathogen which can be transmitted to humans through direct contact with animals and consumption of contaminated food, water, and unpasteurized milk [1].

A gastrointestinal problem commonly caused by *C. jejuni*, especially in children, is called campylobacteriosis [2-4]. CadF as one of the important proteins is a conserved, genus-specific, and 37-kDa outer membrane protein that binds to fibronectin and facilitates bacterial colonization of host cells. CadF could induce massive immune responses, including humoral- and cell-mediated immunity [5-7].

Subunit vaccines usually contain parts of the target microorganisms and are known to be safe and effective vaccines for humans and animals. These vaccines activate both humoral- and cell-mediated immune mechanisms to protect humans against pathogens. However, the identification and prediction of antigenic epitopes by bioinformatics tools are mandatory for the development of a real subunit vaccine [8-11]. Although there are some studies on the development of vaccine candidates based on the outer membrane proteins of *C. jejuni*, little is known about CadF potential to be independently considered in the development of a protective vaccine [12, 13].

This study aimed to analysis CadF protein in order to identify epitope-based peptide candidates and evaluate its proteomic database using bioinformatics tools and servers for developing a new vaccine candidate. Therefore, the work was solely an "*in silico*" study.

Methods

Protein sequences analysis

CadF protein sequences were obtained from both NCBI Protein Data Bank (https://www.ncbi.nlm.nih.gov/protein) and UniProt database (https://www.uniprot.org) in FASTA format. Evolutionary analysis was performed by multiple alignment and phylogenetic tree of the sequences using the ClustalW2 tool (https://www.ebi.ac.uk/Tools/msa/clustalw2) and Molecular Evolutionary Genetics Analysis software Version 7 (MEGA 7).

Protein characterization

The three-dimensional structures and biological functions of the protein were recognized by Phyre2 (www.sbg.bio.ic.ac.uk/phyre2) as an online protein fold recognition server. The protein structures were also analyzed by the PSIPRED server (http://bioinf.cs.ucl.ac.uk/psipred).

Two TMHMM (http://www.cbs.dtu.dk/services/TMHMM) and ProtParam (https://web.expasy.org/protparam) servers were used to predict exo-membrane amino acid sequences and physico-biochemical characteristics of CadF protein.

Allergenicity and antigenicity assessment

The AllerTOP (www.ddg-pharmfac.net/AllerTOP) and AllergenFP (ddg-pharmfac.net/AllergenFP) web servers were used to determine the allergenicity of CadF protein and common peptides. The AllergenFP was databased to obtain a set of options for predicting allergens. The VaxiJen server (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) was also used to forecast the antigenicity of the sequences.

Epitope conservancy assessment

The MHC I and MHC II (Major Histocompatibility Complex) epitopes were analyzed by the IEDB (https://www.iedb.org/), NetCTL (www.cbs.dtu.dk/services/NetCTL), NetMHC (www.cbs.dtu.dk/services/NetMHC), NHLApred (http://crdd.osdd.net/raghava/nhlapred/), SYFPEITHI (www.syfpeithi.de), and MHC2Pred (http://crdd.osdd.net/raghava/mhc2pred/) online servers.

The B cell epitopes were identified using the IEDB, SVMTriP (http://sysbio.unl.edu/SVMTriP/), and BCPREDS (http://ailab.ist.psu.edu/bcpred/) servers by setting a default specificity of 75%; the threshold value of 0.5 was considered for ABCpred (http://crdd.osdd.net/raghava/abcpred) server. Linear and discontinuous B cell epitopes were also predicted by the BepiPred server (http://www.cbs.dtu.dk/services/BepiPred). This server was applied to predict B cell epitopes through the combination of a Hidden Markov Model (HMM) and a propensity scale method. Each epitope identified by these servers was checked to determine the allergenicity and antigenicity properties. The identified common epitopes were analyzed as predicted epitopes, and finally, the best common peptides were selected.

Molecular docking of adopted epitope and alleles

The three-dimensional structures of HLA-A 0101 and HLA-DRB1 0101 alleles were extracted from the Protein Data Bank (https://www.rcsb.org) with the UniProt KB ID: Q5SUL5 and P01911. The PyMOL Molecular Graphics System was used to analyze the three-dimensional structure of the best epitope. Final epitope and alleles were edited by Notepad^{++}, and the interaction between them (epitope/ HLA-A 0101 allele of MHC I and epitope/ HLA-DRB1 0101 allele of MHC II) was assessed with the help of Molecular Virtual Docker and Molecular Virtual Viewer software. Finally, the interfaces between the epitope and alleles were selected based on a grid, computed on three axes, including x: -0.16, y: -17.63, and z: -15.67.

Results

Analysis of *cadF* gene sequences

The complete sequences of CadF protein contained 319 amino acids, and multiple sequence alignment confirmed that this protein was a highly conserved protein among *Campylobacter* species. It was shown to belong to the outer member proteins superfamily (ompA), and an ompA-like domain was identified in the 193-287 position of the protein. The result of the phylogenic tree also confirmed CadF classification in the outer membrane proteins superfamily (data not shown).

Characterization of CadF

Using the ProtParam server, the MV (molecular weight) and pI (isoelectric point) parameters were determined as 35979.04 Da and 5.89, respectively. The aliphatic index was 69.12, and the GRAVY (grand average of hydropathicity) of the protein was -0.679. As a result, the amino acids of CadF protein had hydrophobicity and acidity properties (pI≤7.35). The aliphatic index included alanine, valine, isoleucine, and leucine amino acids, indicating the thermostability of the protein. Moreover, the TMHMM server data analysis results also confirmed that CadF was an outer membrane protein. According to the obtained result from the Phyre2 server, it was predicted that CadF was a stable target.

The PSIPRED server showed the graphical results of secondary structures of the protein, indicating a sheet, helix, and extracellular transmembrane structure. In addition, the Phyre2 server showed the three-dimensional structure of the modeled CadF with a 97% confidence score and 192 known-domain alignments. The structural content included 16% alpha-helix, 41% beta strands, and 16% disordered regions. Also, the prediction of CadF protein showed a binding site at glutamate-histidine-lysine residues and a large amount of metallic heterogenic sections in its structure.

Evaluation of antigenicity and allergenicity

The score of antigenic prediction of CadF protein was calculated as ~0.79 by the VaxiJen server. The results showed that the protein was probably an antigen and could be used for further analysis. The

AllergenFP server data indicated the highest Tanimoto similarity index of 0.82 for the protein; therefore, it could not be an allergen. The AllerTOP server data analysis results also confirmed the finding.

Prediction of T and B cell epitopes

The best score for predicting T cell epitopes was selected from the SYFPEITH, IEDB, NetCTL, NHLAPred, and NetMHC servers. The epitopes of MHC I (A 0101, A 0201, and B 2705) and MHC II (DRB1 0101 and DRB1 0401) were the most frequent epitopes among Iranian alleles that were considered in this study. Using the Kolaskar & Tongaonkar Antigenicity method on the IEDB server, a graph was plotted, suggesting the yellow areas as B cell epitopes (Supplementary File A). According to the obtained results, VLFGADNNV, GLASVLFGA, and LSDSLALRL were the most common epitopes among T and B cells. Further detailed information about the predicted MHC I, II, and B cell epitopes are presented in Tables 1-3, respectively. Overall, the results showed that the best epitope was LSDSLALRL located in 136-144 regions of CadF with antigenic properties and no allergenic specifications. The three-dimensional structure of the final epitope painted by PyMol software is showed in Supplementary File B. Therefore, it was suggested as a candidate vaccine for further analysis.

Table 1. Predicted epitopes of MHC I and their antigenicity and allergenicity properties. The best common peptides are marked in green.

Position Sequence	Allele	Server	VaxiJen	AllerTOP
93GIDVGEKFY	HLA-A 0101	SYFPEITHI	0.017 (Probable NON-ANTIGEN)	NON-Allergen
110YEDFSNAAY			-0.39 (Probable NON-ANTIGEN)	NON-Allergen
61QLEFGLEHY			1.28 (Probable ANTIGEN)	Allergen
25ITPTLNYNY			1.23 (Probable ANTIGEN)	Allergen
79KTTDITRTY			0.56 (Probable ANTIGEN)	Allergen
5FLCLGLASV	HLA-A 0201		0.30 (Probable ANTIGEN)	NON-Allergen
9GLASVLFGA			0.19 (Probable NON-ANTIGEN)	NON-Allergen
13VLFGADNNV			0.19 (Probable NON-ANTIGEN)	NON-Allergen
247ILEGHTDNI			0.50 (Probable ANTIGEN)	Allergen
42NRYAPGVRL	HLA-B 2705		1.43 (Probable ANTIGEN)	NON-Allergen
310RRVDAKFIL			1.45 (Probable ANTIGEN)	NON-Allergen
134FRLSDSLAL			1.35 (Probable ANTIGEN)	Allergen
146TRDQINFNH			0.34 (Probable NON-ANTIGEN)	Allergen
84TRTYLSAIK			-0.27 (Probable NON-ANTIGEN)	Allergen
13FLCLGLASV	HLA-A 0201	IEDB	0.30 (Probable NON-ANTIGEN)	NON-Allergen
251HTDNIGSRA	HLA-A 0101		1.49 (Probable ANTIGEN)	Allergen
310RRVDAKFIL	HLA-B 2705		1.45 (Probable ANTIGEN)	NON-Allergen
9GLASVLFGA	HLA-A 0201		0.19 (Probable NON-ANTIGEN)	NON-Allergen
110YEDFSNAAY	HLA-A 0101		-0.39 (Probable NON-ANTIGEN)	NON-Allergen
15FGADNNVKF	HLA-A 0101	NetCTL	1.00 (Probable ANTIGEN)	NON-Allergen
25ITPTLNYNY			1.23 (Probable ANTIGEN)	Allergen
61QLEFGLEHY			1.28 (Probable ANTIGEN)	Allergen
79KTTDITRTY			0.56 (Probable ANTIGEN)	Allergen
80TTDITRTYL			0.13 (Probable NON-ANTIGEN)	NON-Allergen
110YEDFSNAAY			-0.39 (Probable NON-ANTIGEN)	NON-Allergen
136LSDSLALRL			1.82 (Probable ANTIGEN)	NON-Allergen
136LSDSLALRL	HLA-A 0101	NetMHC	1.82 (Probable ANTIGEN)	NON-Allergen
251HTDNIGSRA			1.49 (Probable ANTIGEN)	Allergen

Table 2. Predicted epitopes of MHC II and their antigenicity and allergenicity properties. The best common peptides are marked in green.

Position Sequence	Allele	Server	VaxiJen	AllerTOP
213EGHFGFDKTTINPTF	HLA-DRB1 0401	IEDB	0.42 (Probable ANTIGEN)	NON-Allergen
212LEGHFGFDKTTINPT			0.29 (Probable NON-ANTIGEN)	NON-Allergen
214GHFGFDKTTINPTFQ			0.31 (Probable NON-ANTIGEN)	NON-Allergen
9GLASVLFGADNNVKF			0.47 (Probable ANTIGEN)	NON-Allergen
10LASVLFGADNNVKFE			0.67 (Probable ANTIGEN)	Allergen
11ASVLFGADNNVKFEI			0.71 (Probable ANTIGEN)	Allergen
216FGFDKTTIN	HLA-DRB1 0101	MHC2Pred	0.33 (Probable NON-ANTIGEN)	Allergen
149QINFNHANH			1.08 (Probable ANTIGEN)	NON-Allergen
87YLSAIKGID			-0.06 (Probable NON-ANTIGEN)	Allergen
305GRADNRRVD			2.78 (Probable ANTIGEN)	NON-Allergen
260YNQKLSERR	HLA-DRB1 0101		1.60 (Probable ANTIGEN)	NON-Allergen
187PQAKCPVEP			0.05 (Probable NON-ANTIGEN)	Allergen
236KVLDENERY			-0.15 (Probable NON-ANTIGEN)	Allergen
36GNLDMDNRY			0.23 (Probable NON-ANTIGEN)	Allergen
85RTYLSAIKGIDVGEK	HLA-DRB 10101	SYFPEITHI	0.13 (Probable NON-ANTIGEN)	NON-Allergen
99KFYFYGLAGGGYEDF			0.72 (Probable ANTIGEN)	NON-Allergen
131GVKFRLSDSLALRLE			2.11 (Probable ANTIGEN)	Allergen
156NHNWVSTLGISFGFG			0.86 (Probable ANTIGEN)	Allergen
37NLDMDNRYAPGVRLG			1.19 (Probable ANTIGEN)	Allergen
149QINFNHANHNWVSTL	HLA-DRB1 0401		0.63 (Probable ANTIGEN)	NON-Allergen
213EGHFGFDKTTINPTF			0.42 (Probable NON-ANTIGEN)	NON-Allergen
276LEKYGVEKSRIKTVG			0.50 (Probable ANTIGEN)	NON-Allergen
11ASVLFGADNNVKFEI			0.71 (Probable ANTIGEN)	NON-Allergen
18DNNVKFEITPTLNYN			1.36 (Probable ANTIGEN)	Allergen

Table 3. Predicted epitopes of B cell and their antigenicity and allergenicity properties. The best common peptides are marked in green.

Position Sequence	Server	VaxiJen	AllerTOP
4IFLCLGLASVLFG	IEDB	0.36 (Probable NON-ANTIGEN)	NON-Allergen
45APGVRLGYHFDD		0.81 (Probable ANTIGEN)	NON-Allergen
136LSDSLAL	ABCpred	0.55 (Probable ANTIGEN)	NON-Allergen
19NNVKFEITPT		1.33 (Probable ANTIGEN)	Allergen
159WVSTLGISFG		0.62 (Probable ANTIGEN)	NON-Allergen
118YDNKSGGFGH		0.79 (Probable ANTIGEN)	NON-Allergen
144LETRDQINFN		0.89 (Probable ANTIGEN)	NON-Allergen
96VGEKFYFYGL		0.52 (Probable ANTIGEN)	Allergen
122SGGFGHYGAG		0.82 (Probable ANTIGEN)	NON-Allergen
170 GGKKEKAVEEVADTRATPQA	BCPREDS	1.39 (Probable NON-ANTIGEN)	Allergen
295NPRSSNDTKEGRADNRRVDA		2.42 (Probable ANTIGEN)	NON-Allergen
214GHFGFDKTTINPTFQEKIKE		0.32 (Probable NON-ANTIGEN)	NON-Allergen
70SDVKYTNTNKTTDITRTYLS		0.69 (Probable ANTIGEN)	NON-Allergen
93GIDVGEKFYFYGLAGGGYED		0.65 (Probable ANTIGEN)	NON-Allergen
115NAAYDNKSGGFGHYGAGVKF		0.91 (Probable ANTIGEN)	Allergen
144LETRDQINFNHANHNWVSTL		0.44 (Probable ANTIGEN)	Allergen
39DMDNRYAPGVRLGYHFDDFW		0.78 (Probable ANTIGEN)	NON-Allergen
237VLDENERYDTILEGHTDNIG		0.33 (Probable NON-ANTIGEN)	NON-Allergen
130AGVKFRLSDSLALRL		2.06 (Probable ANTIGEN)	NON-Allergen
300NDTKEGRADNRRVDAKFILR	SVMTriP	2.04 (Probable ANTIGEN)	Allergen
251HTDNIGSRAYNQKLSERRAK		1.43 (Probable ANTIGEN)	NON-Allergen
2KKIFLCLGLASVLFGADNNV		0.10 (Probable NON-ANTIGEN)	NON-Allergen

Analysis of docking

The bindings of the best epitope to the desired HLA molecules were observed by Molecular Virtual Docker software, and five models were estimated. The proposed models showed the interaction of the epitope side chains with the cavities in the groove of MHC I and II (Supplementary File C). The energies of the

bonding models resulted from the binding of LSDSLALRL peptide to HLA-A 0101 consisted of -26.18, -18.62, -12.77, and -12.56 kcal/mol. The best scores of the peptide docking to HLA-DRB1 0101 were computed as -109.86, -99.52, -98.40, and -85.79 kcal/mol. According to the principles of docking energy evaluation, the model with the most negative docking results was selected as the best model with energies of -26.18 and -109.86 kcal/mol, which were related to HLA-A 0101 of MHC I and HLA-DRB1 0101 of MHC II, respectively.

Discussion

This study was focused on the immunogenic protein CadF to design a hypothetical vaccine through bioinformatics tools which could dramatically reduce the number of in vitro tests. The previous studies have reported some efforts to suggest an effective vaccine against *C. jejuni*. Despite many efforts to make a vaccine, no approved vaccine against *C. jejuni* in humans has been developed as suitable so far [5, 8, 14].

T and B cell epitopes were collected from different servers, and the best epitope was elicited to make an effective vaccine against *C. jejuni*. The present study showed the accurate topology model based on the Phyre2 server, predicting CadF as a stable target. This analysis was done with bioinformatics methods and helped design a novel hypothetical vaccine according to the sequence profile, spatial structure, and dimensions of the protein.

LSDSLALRL epitope was selected as the best potential vaccine candidate without any evidence of allergenicity. The epitope was located in 136-144 regions and could interact with HLA-A 0101 according to the results collected from many above-mentioned servers. In a study by Yasmin et al. (2016), gaining their knowledge of CadF protein based on just IEDB and SYFPEITHI servers, FRLSDSLAL epitope of the protein was suggested as a good choice for vaccine development [15].

It is clear that the epitope selected in this study is fairly matched (77.77%) with the epitope presented by Yasmin et al. (LSDSLALRL and FRLSDSLAL, which are marked by underline). This similarity could support the claim of suitability of the selected epitope for designing an effective vaccine against *C. jejuni*. Based on the AllerTOP server, the presented epitope by Yasmin et al. could probably be estimated as an allergen, while no allergenicity was observed for epitope "LSDSLALRL" in this study.

In addition, CadF is a significant protein for colonization, and maximum attachment could be detected in regions of the fibronectin-binding domain, including phenylalanine-arginine-leucine-serine (FRLS) residues of the protein [16]. Although only 50% of the selected epitope amino acids were identified as the binding site to host cells, multiple servers confirmed that this region had a high score for vaccine development.

According to the aliphatic index, alanine, valine, isoleucine, and leucine amino acids were detected in the protein structure, proposing it as a thermostable protein. These amino acids in thermophilic bacteria, e.g. *C. jejuni*, are significantly higher than that of ordinary proteins [17]. This proposes another advantage

of CadF for the development of an effective vaccine. Heat stability is an important feature in vaccine production, which can

Conclusion

It is suggested that CadF protein of *C. jejuni* could be used to prepare an effective vaccine for disease prevention. However, to predict an actual vaccine without any side effect, knowledge of the pathogenesis and molecular structure of *C. jejuni* needs to be improved through in vitro and in vivo studies in parallel with *in silico* research.

Limitations

There were some limitations in the use of some servers. In addition, due to the limited funding and current facilities of our laboratory, it was not possible to validate the results through in vitro and in vivo projects.

Abbreviations

OmpA: Outer member proteins; CadF: *Campylobacter* adhesion to Fibronectin; Fn: Fibronectin; MHC: Major histocompatibility complex; MV: molecular weight; pI: isoelectric point; kcal/mol: Kilocalorie/mole.

Declarations

Acknowledgment

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Authors' Contributions

MMN, SS, MMN, and BB involved in the management of the project, the analysis of data, and writing up the paper. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent to publish

Not applicable.

Ethics approval and consent to participate

The study was reviewed and approved by Medical Ethics Committee of Qom University of Medical Sciences (Code: IR.MUQ.REC.1399.027).

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Supplementary Files

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