Journal of Current Biomedical Reports
Volume 1, Number 1, 2020
jcbior.com
eISSN: 2717-1906

Original research

# *In silico* prediction of B cell epitopes of the hemolysis-associated protein 1 for vaccine design against leptospirosis

Sakineh Poorhosein Fookolaee[1], Somayyeh Talebishelimaki[1], Mohammad Taha Saadati Rad[2,3], Mostafa Akbarian Rokni[4,*]

[1] *Department of Nursing, Faculty of Nursing and Midwifery, Islamic Azad University, Sari, Iran*
[2] *Young Researchers and Elite Club, Sari Branch, Islamic Azad University, Sari, Iran*
[3] *Department of Medicine, Sari Branch, Islamic Azad University, Sari, Iran*
[4] *Department of Nursing, Faculty of Nursing & Midwifery, Babol University of Medical Sciences, Babol, Iran*

## Abstract

Leptospirosis is known as a zoonotic disease of global importance originated from infection with the spirochete bacterium *Leptospira*. Although several leptospirosis vaccines have been tested, the vaccination is relatively unsuccessful in clinical application despite decades of research. Therefore, this study was conducted to predict B cell epitopes of the hemolysis-associated protein 1 (Hap1) for vaccine design against leptospirosis. For prediction of linear epitopes, the sequence of extracellular region of Hap1 was submitted to ABCpred, BCPREDs, Bcepred, Bepipred and Ellipro servers. DiscoTope 2.0 and B-pred servers were used for prediction of conformational epitopes from the entire PDB structure of Hap1 that obtained from the homology modeling method. Further analysis for solvent accessible areas and relative solvent accessibility of all the residues on the PDB structures using Naccess program and NetSurfP server defined that predicted conformational B cell epitopes had higher solvent accessible and their residues were exposed on the surface therefore, immunoinformatics analysis showed that hemolysis-associated protein 1 can properly stimulate the B cells immune responses.

**Keywords:** Leptospirosis; Hemolysis-associated protein 1; B cell epitope; *in silico*

## 1. Introduction

Leptospirosis is an infection caused by corkscrew-shaped bacteria called *Leptospira*. Signs and symptoms can range from none to mild such as headaches, muscle pains, and fevers to severe with bleeding from the lungs or meningitis [1]. If it causes jaundice, kidney failure, and bleeding, it is known as Will's disease, and if it causes bleeding in the lungs, it is also known as severe pulmonary hemorrhage syndrome.[2]. *Leptospira* can be transmitted by both wild and domestic animals, and up to 10 different genetic types of it can cause disease in humans. The most common animals that spread the disease are rodents [3]. The identification of the common immunogenic proteins of *Leptospira* would be a major step toward the development of purer, better-defined, and probably more-efficient vaccines. Such vaccines would provide cross-protection against a wide range of pathogenic *Leptospira* strains [4] Outer membrane proteins such as LipL21 and LipL32, LipL41 are expressed only in pathogenic species of *Leptospira* [5]. Recent data indicated that hemolysis-associated protein 1 (Hap1) (Figure 1) could be a good candidate for developing a new generation of vaccines able to

---

[*] **Corresponding author:**
Dr. Mostafa Akbarian Rokni, Ph.D
Department of Nursing, Faculty of Nursing & Midwifery,
Babol University of Medical Sciences, Babol, Iran
Tel/Fax: +98 935 8462251
Email: akbarianmostafa1357@gmail.com
https://orcid.org/0000-0002-9198-6818

Received: July, 28, 2020
Accepted: August, 18, 2020

© The Author(s) 2020

induce broad protection against leptospirosis disease [6]. It has been suggested that this protein is only produced by pathogenic leptospires and Hap1 vaccination induces significant protection compared to similar OmpL1 based vaccination [6]. Active immunotherapy with the peptide vaccines which are designed to be chimeric with multi-epitopes of B cells and T helper cells can induce the generation of an adaptive immune response [7]. Several experimental techniques are currently available for the selection of suitable B cell epitopes. The experimental approaches applied for detecting immunogenic regions are often laborious and resource-intensive. Computational techniques for predicting B cell epitopes are fast, scalable, and cost-effective, focusing on experimental experiments and a better understanding of antigen-antibody interaction [8]. Recent researches have shown there are limitations to the current epitope prediction methods. Therefore, enhancing the reliability of computational B cell epitope prediction methods remains a major challenge in computational vaccinology [9]. Nevertheless, prediction results produced by multiple computational tools could be used to gain a consensus result. Basically, the recognition of either small discrete T-cell epitopes or large conformational epitopes recognized by soluble antibodies and B cells is the key molecular event for the immune response to pathogens. B cell epitopes can be classified into two types: linear (continuous) and conformational (discontinuous). While linear epitopes comprise of continuous residues in the sequence, conformational epitopes are composed of amino acids that are not neighboring in primary sequence and are brought into close proximity in the folded protein structure [10]. Localization of these epitopes is of clinical interest for the development of diagnostic tools, vaccines, and cancer immunotherapies. Many attempts have been made for predicting the antigenic sites from certain features of proteins' primary structures. Different parameters such as static accessibility, hydrophilicity, and mobility of the short segments in polypeptide chains have been associated with the position of continuous epitopes in proteins [11]. The present research aimed to predict new B cell epitopes for the Hap1. Combination approaches were used by combining results from the sequence and the structure-based methods and the solvent-accessible surface area calculating tools. Furthermore, we used PEPOP 2.0 application to predict new conformational epitopes from PDB structure of Hap1.

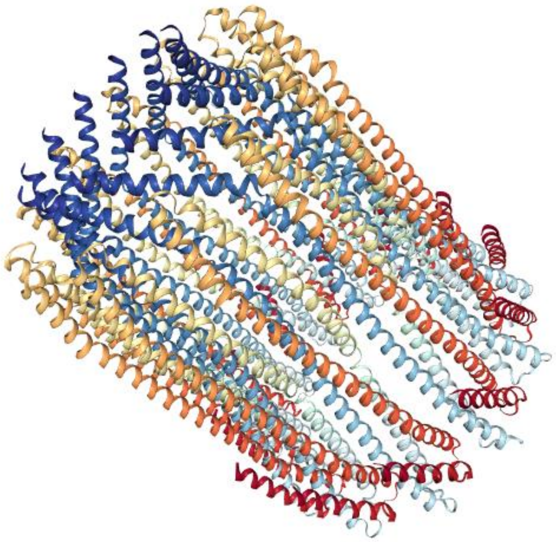

Figure 1. Tertiary structure of Hap 1. In this figure the protein is shown in the third structure

## 2. Materials and Methods

### 2.1. Retrieval of protein sequences

All sequences of Hap1 were retrieved from UniProt (www.uniprot.org) in FASTA format.

### 2.2. Linear B cell epitopes prediction

For the prediction of linear epitopes, the sequence of Hap1was submitted to ABCpred, BCPREDs, Bcepred, Bepipred, and Ellipro servers [9, 12]. The hidden Markov model, Thornton's method, Support Vector Machine classifiers, Recurrent Neural Network, and physicochemical properties of amino acids were applied to predict linear B cell epitopes. Only, the linear peptides which were predicted frequently by 3 or more servers were selected.

### 2.3. Conformational B cell epitope prediction

DiscoTope 2.0 and B-pred servers were used for the prediction of conformational epitopes from the entire PDB structure of Hap1 that obtained from the homology modeling method. The DiscoTope method incorporates a new spatial neighborhood description and a half-sphere exposure as a surface measure based on the protein structure and epitope propensity scores and predicts residues that can be involved in B-cell epitopes. B-pred is a web-based platform for scoring and predicting B-cell epitopes based on the structures of the potential immunological proteins. The method scores the peptides set of a protein-based on the average solvent exposure, by a filter on filtering the average local model quality for each peptide [13].

## 3. Results

The predicted linear B cell epitopes of the HAP1 are shown in Table 1. The predicted B cell epitopes were ranked according to their score obtained by a trained recurrent neural network. A higher score of peptide means a higher probability to be an epitope. As listed in Table 1, peptides were predicted by 5 servers used in the current study. The peptide "TGEIGEPGDGDLVSDA" had the highest score compared to other epitopes. These peptides reside in 37-210 regions of the Hap1. Some studies on the vaccine are shown in the Table 2.

Discotope and B-pred predicted 167 B-Cell epitope residues out of 3420 total residues corresponding to conformational epitopes, approximately located in the region of linear B cell epitopes (Table 3). Further analysis for solvent accessible areas and relative solvent accessibility of all the residues on the PDB structures using the Naccess program and NetSurfP server defined that predicted conformational B cell epitopes had higher solvent accessible and their residues were exposed on the surface.

## 4. Discussion

Although the majority of B cell epitopes appear to be conformational, most of the computational methods focused on the prediction of sequential epitopes. Linear epitope prediction approaches can be classified as propensity scale methods, improved propensity scale methods and machine learning methods [23]. If the tertiary structure of an antigen is known, there are improved methods for identifying conformational B cell epitopes. Examples are Discotope web server and PEPOP. These are based on features like amino acid propensity scales and solvent accessibility [24]. In this study, linear and conformational B cell epitopes of Hap1 were predicted using both primary sequence and tertiary structure. Based on combination approaches and considering lowest identity with the IR and frequently prediction using several tools, the best peptides were the linear B cell epitope TGEIGEPGDGDLVSDA (Table 1) and conformational B cell epitopes (Table 2). In addition, PEPOP also predicted 167 new conformational peptides.

Table 1. Predicted linear B cell epitopes using various servers.

| Rank | Sequence | Start position | Score |
|---|---|---|---|
| 1 | TGEIGEPGDGDLVSDA | 101 | 0.96 |
| 2 | GSVINYYGYVKPGQAP | 54 | 0.94 |
| 3 | YRISFTTYKPGEVKGS | 210 | 0.89 |
| 3 | DDGDDTYKEERHNKYN | 164 | 0.89 |
| 4 | GQAPDGLVDGNKKAYY | 66 | 0.88 |
| 5 | EDTIPGTNETVKTLLP | 37 | 0.85 |
| 5 | RIKIPNPPKSFDDLKN | 183 | 0.85 |
| 5 | FDTWIRVERMSAIMPD | 131 | 0.85 |
| 6 | PEEKSMPHWFDTWIRV | 122 | 0.84 |
| 7 | YGYVKPGQAPDGLVDG | 60 | 0.83 |
| 7 | NETVKTLLPYGSVINY | 44 | 0.83 |
| 7 | RHNKYNSLTRIKIPNP | 174 | 0.83 |
| 8 | ASVGLLFPPGIPGVSP | 228 | 0.81 |
| 9 | AVIAEMGVRMISPTGE | 88 | 0.80 |
| 9 | AKAAKAKPVQKLDDDD | 149 | 0.80 |
| 10 | QKQAIAAEESLKKAAS | 253 | 0.78 |

Table 2. Some studies on the vaccine are shown in the table below

| Vaccine | Antigen | Adjuvant | Vector | Resistance marker | Promoter | Leader sequence | Animal model | Immunization | | Challenge Serovar/Dose | Vaccinated/Control | | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Dose | Route | | Total No. | % Survival | |
| DNA | Hap1 | NA | pcDNA3.1 | Neomycin | Human cytomegalovirus (CMV) | – | Gerbils | 2 | 100 mg/IM | Can/$10^7$ | 15/20 | 60/35 | [14] |
| DNA | OmpL1 | NA | pcDNA 3.1 | Neomycin | Human cytomegalovirus (CMV) | – | Hamsters | 3 | 100 mg/IM | Pom/$10^3$ | 6/6 | 33/0 | [15] |
| DNA | LipL32 | NA | pTARGET | Ampicillin | Human cytomegalovirus (CMV) | – | Mice | 2 | 100 mg/IM | NA | 5/5 | NA | [16] |
| DNA | LipL21 | NA | pcDNA 3.1 | Neomycin | Human cytomegalovirus (CMV) | – | Guinea Pig | 2 | 100 mg/IM | Lai 56601 | 8/8 | 100/100 | [17] |
| DNA | LipL32 | NA | pVAX1 | Kanamycin | Human cytomegalovirus (CMV) | – | Mice | 3 | 50 mg/IM | NA | 10/10 | NA | [18] |
| DNA | LipL32–41-OmpL1 | NA | pVAX1 | Kanamycin | Human cytomegalovirus (CMV) | – | Mice | 3 | 50 mg/IM | NA | 10/10 | NA | [18] |
| DNA | LemA | Alhydrogel | pTARGET | Neomycin | Human cytomegalovirus (CMV) | – | Hamsters | 2 | 100 mg/IM | Cop/$10^1$ | 8/6 | 62.5/0 | [19] |
| PB | LemA | Alhydrogel | pTARGET | Neomycin | Human cytomegalovirus (CMV) | – | Hamsters | 2 | 100 mg/IM | Cop/$10^1$ | 8/6 | 87.5/0 | [19] |
| DNA | LipL32 | Poly I:C | pVITRO1 | Neomycin | EF–1a | VZV gE signal peptide | Mice | 3 | 20 mg/IM or EP | NA | 6/6 | NA | [20] |
| DNA | OmpL37 | Alhydrogel | pTARGET | Neomycin | Human cytomegalovirus (CMV) | – | Hamster | 2 | 100 mg/IM | Cop/$10^3$ | 6/6 | 0/0 | [21] |
| PB | OmpL37 | Alhydrogel | pTARGET | Neomycin | Human cytomegalovirus (CMV) | – | Hamster | 2 | 100 mg/IM | Cop/$10^3$ | 6/6 | 25/8.3 | [21] |
| DNA | LigBrep | Alhydrogel | pTARGET | Neomycin | Human cytomegalovirus (CMV) | – | Hamster | 2 | 100 mg/IM | Cop/$10^1$ | 5/5 | 40/0 | [22] |
| PB | LigBrep | Alhydrogel | pTARGET | Neomycin | Human cytomegalovirus (CMV) | – | Hamster | 2 | 100 mg/IM | Cop/$10^1$ | 6/5 | 83.3/0 | [22] |

Table 3. Conformational B-cell epitopes from full-length protein using different servers

| Epitope | Position |
|---|---|
| ASP, GLU, THR, ILE, LYS, GLU | 14, 14, 30, 24, 8, 20 |
| SER | 15 |
| PHE, GLN | 13, 27 |
| PRO | 9 |
| LYS, ASN | 8, 15 |
| LEU, LYS | 22, 6 |
| ASP, GLU, THR, ILE, LYS, GLU | 15, 14, 30, 24, 7, 20 |
| SER | 23 |
| SER | 15 |
| PHE | 24 |
| PRO | 8 |
| LYS, ASN | 8, 15 |
| LEU, LYS | 22, 7 |
| ASP, GLU, THR | 14, 14, 30 |
| LYS, GLU | 8, 20 |
| SER | 20 |
| PHE, GLN | 24, 13 |

The predicted epitopes by PEPOP are widely distributed within the CRR (Cysteine-rich region) domain and often partly overlapped, consistent with the view that PEPOP predicted segmented epitopes and the CRR domain displayed a mosaic of overlapping epitopes. Considering less identity with the IR and high RSA (Relative solvent accessibility) score conformational epitopes can be suitable for further experimental tests. In the present work, new conformational epitope and a linear B cell epitope were predicted using various bioinformatics analyses. In conclusion, findings of the present work, using the bioinformatics analyses could be used in mAbs (monoclonal antibodies) production, vaccine design and the diagnostic tools. In addition, the current *in silico* approaches are reducing time and minimizing the total number of necessary tests to find possible and proper epitopes. In the next step, synthesis of determined peptides in vitro and in vivo experimental studies are essential for assurance of the predicted epitopes. Due to the prevalence of this disease in Iran, especially in the northern regions of the country, which have paddy fields and agricultural lands and many people are at risk of contracting this disease, the preparation and design of vaccines seems necessary.

In this research, we used immunoinformatics methods to predict appropriate vaccine against *Leptospira*. Selection of suitable epitopes of the Hap1 as antigens, and utilizing them for raising mAbs against the Hap1, with ability of *Leptospira* inhibition would be beneficial in treatment of leptospirosis. To the best of our knowledge, for the first time, in this study the linear and conformational B cell epitopes of the Hap1 were predicted, screened and assessed using the well-known bioinformatics comprehensive analyses. All sequences were joined to each other by proper linkers. Epitopes were evaluated as non-allergenic, antigenic, soluble, with safety and efficacy. These predicted epitopes might be used to design a vaccine against *Leptospira*, could be validated in model hosts to verify their efficacy as vaccine.

## Author Contributions

All authors contributed equally to this manuscript, and approved the final version of manuscripts.

## Conflict of Interests

The authors declare that they have no conflicts of interest.

## Ethical declarations

Not applicable.

## Financial Support

None.

## References

1. Lane AB, Dore MM. Leptospirosis A clinical review of evidence based diagnosis, treatment and prevention. World J Clin Infect Dis. 2016; 6(4):61-66.
2. Palaniappan RU, Ramanujam S, Chang Y-F. Leptospirosis: pathogenesis, immunity, and diagnosis. Curr Opin Infect Dis. 2007; 20(3):284-92.
3. Adler B, de la Peña Moctezuma A. Leptospira and leptospirosis. Vet Microbiol. 2010; 140(3-4):287-96.
4. Wang Z, Jin L, Węgrzyn A. Leptospirosis vaccines. Microb Cell Fact. 2007; 6:39.
5. Hoke DE, Egan S, Cullen PA, Adler B. LipL32 is an extracellular matrix-interacting protein of Leptospira spp. and Pseudoalteromonas tunicata. Infect Immun. 2008; 76(5):2063-9.
6. Branger C, Sonrier C, Chatrenet B, Klonjkowski B, Ruvoen-Clouet N, Aubert A, et al. Identification of the hemolysis-associated protein 1 as a cross-protective immunogen of Leptospira interrogans by adenovirus-mediated vaccination. Infect Immun. 2001; 69(11):6831-8.
7. Powell, Michael F., Newman, Mark J. (Eds.). Vaccine design: The subunit and adjuvant approach: Springer; 2012.
8. Blythe MJ, Flower DR. Benchmarking B cell epitope prediction: underperformance of existing methods. Protein Sci. 2005; 14(1):246-8.
9. Saha S, Raghava GP. Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. Proteins. 2006; 65(1):40-8.

10. Ahmad TA, Eweida AE, Sheweita SA. B-cell epitope mapping for the design of vaccines and effective diagnostics. Trials Vaccinol. 2016; 5:71-83.

11. El-Manzalawy Y, Honavar V. Recent advances in B-cell epitope prediction methods. Immunome Res. 2010; 6 Suppl 2(Suppl 2):S2.

12. Saha S., Raghava G.P.S. BcePred: Prediction of Continuous B-Cell Epitopes in Antigenic Sequences Using Physico-chemical Properties. In: Nicosia G., Cutello V., Bentley P.J., Timmis J. (eds) Artificial Immune Systems. ICARIS 2004. Lecture Notes in Computer Science, vol 3239. Springer, Berlin, Heidelberg.

13. Kringelum JV, Lundegaard C, Lund O, Nielsen M. Reliable B cell epitope predictions: impacts of method development and improved benchmarking. PLoS Comput Biol. 2012; 8(12):e1002829.

14. Branger C, Chatrenet B, Gauvrit A, Aviat F, Aubert A, Bach JM, et al. Protection against Leptospira interrogans sensu lato challenge by DNA immunization with the gene encoding hemolysin-associated protein 1. Infect Immun. 2005; 73(7):4062-9.

15. Maneewatch S, Tapchaisri P, Sakolvaree Y, Klaysing B, Tongtawe P, Chaisri U, et al. OmpL1 DNA vaccine cross-protects against heterologous Leptospira spp. challenge. Asian Pac J Allergy Immunol. 2007; 25(1):75-82.

16. Nascimento IP, Leite LC. Recombinant vaccines and the development of new vaccine strategies. Braz J Med Biol Res. 2012; 45(12):1102-11.

17. He HJ, Wang WY, Wu ZD, Lv ZY, Li J, Tan LZ. Protection of guinea pigs against Leptospira interrogans serovar Lai by LipL21 DNA vaccine. Cell Mol Immunol. 2008; 5(5):385-91.

18. Feng CY, Li QT, Zhang XY, Dong K, Hu BY, Guo XK. Immune strategies using single-component LipL32 and multi-component recombinant LipL32-41-OmpL1 vaccines against leptospira. Braz J Med Biol Res. 2009; 42(9):796-803.

19. Hartwig DD, Forster KM, Oliveira TL, Amaral M, McBride AJ, Dellagostin OA. A prime-boost strategy using the novel vaccine candidate, LemA, protects hamsters against leptospirosis. Clin Vaccine Immunol. 2013; 20(5):747-52.

20. Buaklin A, Palaga T, Hannaman D, Kerdkaew R, Patarakul K, Jacquet A. Optimization of the immunogenicity of a DNA vaccine encoding a bacterial outer membrane lipoprotein. Mol Biotechnol. 2014; 56(10):903-10.

21. Oliveira TL, Grassmann AA, Schuch RA, Seixas Neto AC, Mendonça M, Hartwig DD, et al. Evaluation of the Leptospira interrogans Outer Membrane Protein OmpL37 as a Vaccine Candidate. PLoS One. 2015; 10(11):e0142821.

22. Forster KM, Hartwig DD, Oliveira TL, Bacelo KL, Schuch R, Amaral MG, et al. DNA prime-protein boost based vaccination with a conserved region of leptospiral immunoglobulin-like A and B proteins enhances protection against leptospirosis. Mem Inst Oswaldo Cruz. 2015; 110(8):989-95.

23. Larsen JE, Lund O, Nielsen M. Improved method for predicting linear B-cell epitopes. Immunome Res. 2006; 2:2.

24. Haste Andersen P, Nielsen M, Lund O. Prediction of residues in discontinuous B-cell epitopes using protein 3D structures. Protein Sci. 2006; 15(11):2558-67.