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Introducing and Bioinformatic Evaluation of a Chimeric and Effective Immunogen Construct for Designing a Recombinant Vaccine against *Brucellosis*.

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ABSTRACT

Background and Aim: Malt fever is a zoonotic disease caused by *Brucellosis* bacteria (an obligate intracellular pathogen) which is highly contagious. Usually, in common vaccines to deal with this pathogen, the whole body of the bacteria is used. Therefore, it causes many side effects. Nowadays, the use of subunit vaccines with the aim of eliminating pathogenic parts and strengthening immunogenic parts has been highly regarded.

Materials and Methods: In this study, the epitopes of L7/L12, BLS and bp26 antigens of *Brucella* bacteria were used to design the recombinant immunogenic structure. For this purpose, the reliable and online IEDB and IFNepitope servers were used to predict the epitopes of each antigen. After identifying the strongest epitopes of these antigens, structural engineering was performed using epitopes, peptide linkers and HBHA as a molecular adjuvant. Evaluation of the physicochemical properties, secondary structure, tertiary structure, antigenicity and allergenicity of the protein sequence of the recombinant construct were performed by ProtParam, SOPMA, I-TASSER, VaxiJen and AllerTOP servers, respectively. Finally, after predicting the second and third structure of the recombinant structure by SOPMA and I-TASSER servers, the protein-protein interaction between the HBHA molecule present in the recombinant structure and the TLR4/MD-2 receptor was investigated using ClusPro online server.

Results: Based on the results, the recombinant structure was successfully designed in two adjuvant (N-terminal) and epitopic (C-terminal) domains using rigid EAAAK linker. Other evaluations showed that the recombinant structure with a molecular weight of 36256.13 Da and an isoelectric point of 8.37, has an aliphatic index of 77.10 and a GRAVY index of -0.613. Also, based on this instability index, this protein is classifiedas a stable protein. Further investigations revealedthat this structure was reported to be a non-allergenic structure with an antigenicity score of 0/8287. The results of protein-protein docking showed that the interaction between HBHA molecule and TLR4/MD-2 receptor with 62 cluster members and the lowest binding energy of -858.8 kcal/mol will be done successfully.

Conclusion: Therefore, the epitope-based recombinant structure can be introduced as a successful structure for engineering subunit vaccines.

Keywords: Bioinformatics, Subunit Vaccine, Brucellosis, Recombinant Protein, Epitope, HBHA

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