Abstract

Introduction: *Mycobacterium tuberculosis* has been known as a main cause of tuberculosis infection for many years. Regardless of the fact that global BCG vaccination has resulted in a substantial reduction in new cases, the problem still remains unresolved. The necessity of disease prevention, and the BCG vaccine's variable efficiency (0-80%) and the limitations of the live attenuated vaccines, have spurred researchers to develop alternative vaccines.

Methods: The sequences of five Rv0888, Rv2645, Rv3841, Rv3874, Rv3875 antigens, as well as Heparin-Binding Haemagglutinin as an adjuvant, were retrieved in this study. Different epitopes were identified employing various databases, and the selected epitopes and adjuvant were linked together using appropriate linkers. Then, allergenicity, antigenicity, solubility and physico chemical parameters of the designed vaccine were analyzed. The study of 3D structural modeling, refinement, and validation was performed. Finally, studies of molecular docking, reverse translation, and codon optimization were performed.

Results: A vaccine with a length of 704 amino acids was designed by selecting the appropriate epitope sequences. Allergenicity, antigenicity, solubility and physico chemical parameters studies have shown that the protein is antigenic, non-allergenic, soluble and stable. Moreover, when the refined 3D structure was compared to the original model, it was indicated that the 3D structure had improved and the potential mistakes were minimized. The vaccine can bind appropriately to the Toll-like receptor 4, as according molecular docking investigations. Codon optimization revealed that the designed protein vaccine can be over-expressed in the E. coli host in vitro.

Conclusion: The fundamental purpose of this study is to use bioinformatics tools to design an appropriate subunit vaccine. As a result, a subunit vaccine consisting of eight epitopes from five *Mycobacterium tuberculosis* antigens which was linked to HBHA adjuvant by GPGPG linker was designed. Various evaluations in this study as well as molecular docking between vaccine and TLR-4 showed that the designed vaccine can be a good candidate for tuberculosis and in practice, it is hoped that satisfactory results may be achieved.

Keywords: Tuberculosis, Epitope, Vaccine, Mycobacterium tuberculosis, Bioinformatics, Adjuvant