In-Silico Development of Multi-epitope Antigens as Candidate Sero-diagnostic Markers for Diagnosis of Leptospirosis

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Leptospirosis is a bacterial zoonosis with a worldwide distribution. Laboratory tests are required for disease confirmation as clinical manifestations of leptospirosis are non-specific. Accurate laboratory diagnosis is challenging due to the high species diversity of the Leptospiragenus. This study represents a series of in silico approaches to design genus-specific multi-epitope antigens as candidate sero-diagnostic markers. A total of 1556 genes were analyzed from the genome sequence of *Leptospira interrogans*(*L. interrogans*) serovar Lai strain 56601. SignalP and LipoP programs were used to predict the outer membrane protein (OMP) localization. PRED-TMBB and TMHMM programs were used to predict the transmembrane (TM) β-barrel structure and reconfirmed by RaptorX and SWISS-MODEL software. Protein conservation and B-cell epitope analysis were performed using pBLAST and IEDB webservers, respectively. Epitopes were assembled into single proteins using flexible linkers to form multi-epitope antigens. Antigenicities of multi-epitopes and OMPs were comparatively analyzed using ANTIGENpro tool. A total of 19 TM β-barrel OMPs were found to be conserved across the *Leptospira* genus. Thirty-three genus-specific linear B-cell epitopes were predicted. Antigenicity analysis showed that some multi-epitope antigens to bear higher B-cell reactivity than native OMPs. In conclusion, the use of multi-epitope antigens in sero-diagnostic assays may result in higher specificity than native antigens and can be used to diagnose leptospirosis regardless of the infecting Leptospira species. These markers may be particularly useful in the diagnosis of leptospirosis in geographical areas with high species diversity. The study provides a useful starting point for studies investigating the potential use of multi-epitope antigens as sero-diagnostic markers.

Keywords: Leptospira, outer membrane proteins, ELISA, multi-epitope antigen