Purification and Immunological Characterization Evaluation of Recombinant Exotoxin A (Domains I, II) Protein isolated from *Pseudomonas aeruginosa*

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Background & Objectives: *Pseudomonas aeruginosa (P.aeruginosa)* is a commonly isolated pathogen from nosocomial infections worldwide this bacterium has evolved several antibiotic resistance mechanisms and antibiotics have failed in treatment of *P. aeruginosa* infections. Hence, immunoprophlaxy and immunotherapy may be an effective method for control and treatment of *P. aeruginosa* infections. In the present study, the immunological properties of exotoxin A (domain I,II) was evaluated

Methods: The DNA encoding for domains I and II of Exotoxin A was amplified by PCR and cloned in PET22b expression vector. The construct was then transformed in to *E. coli* BL21 and the protein expression was evaluated by SDS-PAGE method. The Ni-NTA affinity chromatography was used for recombinant protein purification. The identity of recombinant protein was confirmed by western blotting using anti exotoxin A antibody (Sigma). The reactivity of recombinant exotoxin A with sera from *P.aeruginosa* infected patients was used for evaluation of immunogenicity.

Results: The electrophoresis of PCR products on 1% agarose showed 1722 bp band that was in accordance with ExoA (domain I- II) gene size. Sequencing of cloned gene showed that the sequence of ExoA I-II gene was in accordance with Exo A from *P.aeruginosa PAO1*. SDS-PAGE analysis indicated expression of recombinant protein with a molecular weight of approximately 45kDa. Western blotting with anti-exotoxin A antibody (Sigma) verified the identity of recombinantly expressed protein. Immunologic analysis using *P.aeruginosa* infected patients sera showed that the exoA I-II is highly immunogenic protein that identified by patients sera.

Conclusion: Results of this study showed that recombinant exotoxinA (domainI, II) can be tested as a sub-unit vaccine candidate in *P. aeruginosa* infections.

Keywords: *Pseudomonas aeruginosa*; Candidate Vaccine; Exotoxin A; Recombinant Vaccine