

Production and evaluation of multi-epitope recombinant protein of Leishmania infantum in serological diagnosis of visceral leishmaniasis (Kalaazar) by ELISA

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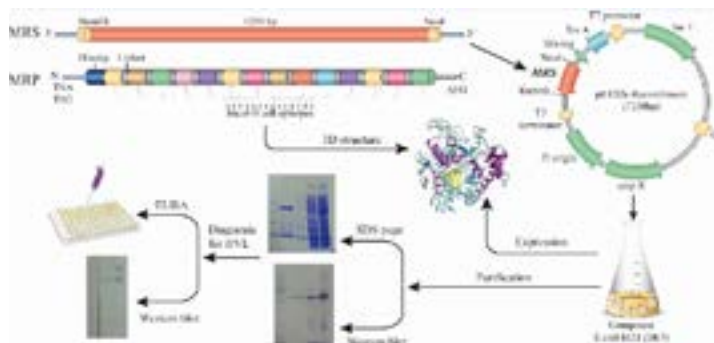
Statement of the Problem: Visceral leishmaniasis, which is Mediterranean in Iran, is caused by leishmania infantum. The importance of this disease is due to the untimely death of the untreated patients, the difficulty in treating patients with immunodeficiency and high numbers of children involved. Therefore, attempts to diagnose rapidly with high sensitivity and specificity and timely treatment are inevitable. The present study was conducted for the first time in Iran to increase the sensitivity and specificity of the diagnostic ELISA test for human visceral leishmaniasis using a multi-epitope antigen.

Methodology & Theoretical Orientation: A gene fragment composed of eight different epitopes from the major antigens of Leishmania Infantum was developed and sent to biomatic company for synthesizing. The Multi-epitope recombinant sequence was cloned and expressed in pET 32a and E. Coli BL21 (DE3) respectively. The antigen was identified by the SDS page and then verified with the Western blot. Purification was performed by affinity chromatography Ni-NTA resin (sigma). Finally, pure MRP was coated in microtiter plate and the sensitivity & specificity detection of anti-leishmaniasis antibodies in the serum of patients was evaluated.

Findings: Intact cloned plasmid with 7kb weight, intact uncloned plasmid with 5.5kb and as expected the insert sequence fraction with 1.2 kb on a 1% agarose gel was detected. Then, bacterial expression lysate on acrylamide gel 12%, was shown recombinant antigen band with 63 KDa size markers. The protein band was confirmed in the next step by western blot with the same molecular weight. Sensitivity and specificity of the antigen were calculated 93.1% and 77.14% respectively in ELISA serodiagnosis test.

Conclusion & Significance: MRP contained epitopes of several important L. infantum antigens was successfully expressed in the prokaryotic system and results of its potential serodiagnosis of HVL were well documented. According to reaction between the MRP and the specific IgG in humans, it could be concluded that the main objectives of the present study are best achieved. More optimization has been postponed for future study due to the time limitation. Further studies are required to evaluate the immunogenicity in animal models and to verify the immuno-reactivity of MRP as a new diagnostic antigen.

Keywords: Visceral leishmaniasis, Human, Iran, serodiagnosis, ELISA, Multi-epitopes, B cell epitopes, Recombinant antigens, MRP



Recent Publications

1. Jameie F, Dalimi A, Pirestani M, et al. Development of a Multi-Epitope Recombinant Protein for the Diagnosis of Human Visceral Leishmaniasis. *Iranian Journal of Parasitology*. 2021 Jan; 16(1):1.
2. Heidari S, Hajjarian H, Kazemi B, et al. Identification of immunodominant proteins of *Leishmania infantum* by immunoproteomics to evaluate a recombinant multi-epitope designed antigen for serodiagnosis of human visceral leishmaniasis. *Experimental Parasitology*. 2021 Mar 1; 222:108065.
3. Jameie F, Dalimi A, Pirestani M, et al. Detection of *Leishmania infantum* Infection in Reservoir Dogs Using a Multiepitope Recombinant Protein (PQ10). *Archives of Razi Institute*. 2020; 75(3):327.
4. Vale DL, Machado AS, Ramos FF, et al. Evaluation from a B-cell epitope-based chimeric protein for the serodiagnosis of tegumentary and visceral leishmaniasis. *Microbial Pathogenesis*. 2022 Jun 1; 167:105562
5. Machado AS, Ramos FF, Oliveira-da-Silva JA, et al. An immunoproteomics approach to identify *Leishmania infantum* proteins to be applied for the diagnosis of visceral leishmaniasis and human immunodeficiency virus co-infection *Parasitology* 2020; 147(9):932-9.

Biography

Marzieh Taherzadeh has recently completed her PhD course and work at Bushehr medical science university now. During her educational and research experience in the field of parasitology, she has ended up several successful projects. The present research is the result of her thesis project.

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