Design and construction of a new recombinant fusion protein (2b2t+EPC1) and its assessment for serodiagnosis of cystic echinococcosis.

Fathi S1, Jabouzani F1, Hosseini SH1, Najafi A2, Darabi E3, Koochmar F4

Author information
1 Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2 Molecular Biology Research Center, Systems Biology and Poisoning Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran.
3 Department of Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
4 Paramedical Faculty, Golestan University of Medical Sciences, Gorgan, Iran.

Abstract
The immunodiagnostic tests for cystic echinococcosis (CE) are mostly serological tests based on ELISA that use hydatid cyst antigens for primary screening because of its simple preparation and availability. The challenge to develop new serological methods (as compared to those based on the hydatid cyst fluid antigens) to meet the gold standard remains. Appropriate sources of antigenic material are necessary for application to improve the efficacy of immunodiagnostic tests at a population level. In the current study, a fusion protein containing the coding sequence of antigen B2t and two sequences of EPC1 antigen with some modifications was reconstructed. Using bioinformatics tools, these sequences were joined together by applying the sequence of a rigid α-helix-forming linker to obtain an appropriate structure of a fusion protein. Synthetic recombinant fusion protein was expressed using pET28a as a vector and evaluated by indirect ELISA test for sera from patients with hepatic CE and other parasitic infections. The sensitivity of the fusion protein was lower (96.46%) than the available ELISA kit (96.15%). However, the differences in sensitivity were not statistically significant as compared to the recombinant fusion peptide with the commercial kit (p = 0.269). The specificity of the recombinant fusion protein (95.45%) was not significantly lower than the commercial kit (96.56%; p = 1.000). Moreover, surprisingly there was no difference in the cross-reactivity values of performance between the recombinant-ELISA and commercial kit. The positive and negative predictive values of the recombinant antigen were achieved as 92% and 93.33%, respectively, while for the commercial kit, they were obtained as 94.33% and 97.70%, respectively. In conclusion, as an early evaluation of these antigens the performance of our recombinant fusion protein in ELISA is relatively promising. Although, it seemed that this peptide with specific antigenic epitopes might be more appropriate for the serological evaluation of CE by use of bioinformatics tools, our findings showed that cross-reactions and a negative reaction could occur in clinical performance. This fusion protein may have utility for diagnosis in humans, but further evaluation is needed using the WHO ultrasound classification for CE.

KEYWORDS: Antigen B2 (2b2t); P1 and P5 peptide of EPC1; cystic echinococcosis; diagnosis; recombinant fusion protein

PMID: 29656723 DOI: 10.1111/apm.12833
Indexed for MEDLINE