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Toxoplasma gondii proteins: development of bioinformatics analysis for vaccine design against toxoplasmosis

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Motivation and Aim: Toxoplasmosis is one of the ubiquitous zoonotic diseases in the world, caused by intracellular parasite *Toxoplasma gondii*. This parasite causes infection in humans described as congenital and or postnatal transmission. Due to the lack of appropriate vaccines and drug to prevent toxoplasmosis, this study intends to develop bioinformatics screening of epitope-based vaccine.

Methods and Algorithms: The protein sequences of *T. gondii* candidate antigens were retrieved in FASTA format via a high-quality web server of protein sequence called Vaxquery, available at http://www.violinet.org/vaxquery/ for further analysis. Antigens have chosen for this study included; MIC3, GRA5, p30, ROP8, ROP2, SAG2, MIC13, hsp70, and OMPDC. The immunogenecity of the selected antigens were predicted using VaxiJen v2.0 server (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html). Non-toxic epitopes were chosen and further analysis was done with antigen that had the highest immunogenicity and lack allergenicity. ABCpred online server (https://webs.iiitd.edu.in/raghava/abcpred/ABC_submission.htm) was employed to predict the 16 amino acids linear B cell epitopes with a specificity threshold of 0.75 %. We used overlaps filter. This server recruits a combination of two approaches (subsequence kernel and support vector machine) by 74.57 % performance accuracy. Epitopes with e highest immunogenicity and lack of allergenicity and toxicity are considered for further study. Prediction of potential epitopes of 9-mer length for MHC I was provided using Immune Epitope Database (IEDB) accessible at https://www.iedb.org. The screening of antigenicity and allergenicity performed via VaxiJen v2.0 and AllerTOP v2.0 servers. Epitopes with highest immunogenicity, non-allergenicity and non- toxicity, which are high-binder epitopes were selected to design vaccine. Chimer protein design was accomplished for the construction of multi-epitope vaccine. The screening of toxicity and allergenicity of chimer performed via ToxinPred and AllerTOP v2.0 servers respectively. The secondary structure of multi-epitope vaccine, PRABI online server at https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page = /NPSA/npsa_server.html and also, Garnier-Osguthorpe-Robson (GOR IV) method were utilized. SWISS-MODEL online service (https://swissmodel.expasy.org/) was employed for tertiary or three-dimensional (3D) structure of designed vaccine and a

homology modeling technique was recruited. Molecular docking method was performed to assess the binding high affinity of target vaccine construct to respective MHC molecules. The strong binding affinity of the target vaccine towards MHC was validated by these scores.
Results: All the selected conserved protein sequences were subjected to VaxiJen v. 2.0 for prediction of immunogenicity probabilities. Allergenicity prediction carried out by AllerTOP v. 2.0 server and the findings revealed that seven chosen proteins include, GRA5, ROP8, Rop2, MIC13, SOD, hsp70, OMPDC have non-allergic nature and the assessment of toxicity with ToxinPred online server showed that GRA5 had no toxicity and was thus selected for the final vaccine construct. According to the results, one 16-mer potent epitope (GVAGSTRDTGSGGDDS) with a cut-off binding score > 0.9, qualified to be included in the final vaccine sequence. Out of all predicted epitopes, "RDTGSGGDD" was chosen as a target based on the results of epitope screening by using VaxiJen v2.0, AllerTOP v2.0, and ToxinPred servers. The constructed vaccine sequence (274 amino acid residues) validated for immunogenicity, allergenecity, and toxicity and fulfilling all the criteria. The overall investigations revealed that the designed vaccine had appropriate criteria for production.
Conclusion: As current vaccination against *T. gondii* infection is not satisfactory, in the present study, a novel epitope-based vaccine was designed and we employed several bioinformatics tools. GRA4 which express in two infectious stages (tachyzoite and bradyzoite) with strong immunogenicity and non-allergenic nature was selected to obtain better result.