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Review Article

Immunoinformatics Approach for Designing an Epitope-Based Peptide Vaccine against *Treponema pallidum* Outer Membrane Beta-Barrel Protein

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ABSTRACT

Treponema pallidum is a gram negative bacteria and the main cause of syphilis which is classified as chronic inflammatory discompose antecedent transmitted sexually. Syphilis affects the central nervous system and the cardiovascular system, potentially leading to hearing or visual loss, aortic aneurism, stroke-like syndrome, dementia and paralysis. *T. pallidum* has the ability to stimulate adaptive immune and corresponding innate procedures in tissue and blood that might set the era for the HIV's bidirectional transmission. This study expects a real epitope-based vaccine against β-Barrel outer membrane protein of *Treponema pallidum* by immunoinformatics approaches. The sequences were saved from NCBI and a number of prediction tests were undertaken to explore possible epitopes for B-cell, T-cell MHC class I and II. 3D structure of the most hopeful epitopes was illustrated. Two epitopes showed high binding affinity for B-cells, while five epitopes showed high binding affinity for MHCI and MHCII. The results were hopeful to formulate a vaccine with 71.88%population coverage. We expect that these hopeful epitopes helps as a preventive formula for the disease in the future and recommend *in vitro* and *in vivo* studies.

Keywords: Immunoinformatics; *Treponema pallidum*; Outer membrane beta-barrel; Peptide vaccine; Epitope

INTRODUCTION

Syphilis is chronic inflammatory discompose antecedent transmitted sexually by the spirochete *Treponema pallidum* [1-8]. There are three subspecies for *Treponema pallidum* (i.e., subsp. *pallidum* [syphilis], subsp. *endemicum* [bejel], and subsp. *pertenue* [yaws]) and *T. carateum* (pinta) [9,10]. *T pallidum* is vastly motile extracellular bacterium distinguished for its invasiveness, immuno-evasiveness and persistence [11]. Based on data from 2016, the WHO assessments showed that approximately 90 million people who were from China [12], Africa, Asia and the Western Pacific are currently at risk of yaws. Globally, around eleven million [2,10,11,13] rates among commercial sex workers are between 23% and 32%. In the United States [2], people were diagnosed with syphilis in 2008, with mother-to-child conduction occurring in nearly two million pregnancies [14]. Syphilis influence in statement progress to affect the cardiovascular and central nervous systems of infected individuals, potentially leading to aortic aneurism, hearing or visual loss, dementia, paralysis, and stroke-like syndrome. *T. pallidum* has the facility to stimulate corresponding innate and adaptive immune procedures in blood and tissue that could set the period for the bidirectional transmission of HIV [3,15]. The diagnosis of syphilis principally relies on serological testing for reactivity to together treponemal and non-treponemal (cardiolipin) antigens indirect detection examine of cerebrospinal fluid (CSF) of one of the patients can be used. However, they are often insensitive and difficult to entrée [13]. Direct detection methods like dark-field microscopy, direct fluorescent antibody testing, nucleic acid amplification testing [16].

The hidden mechanism within syphilis remains ambiguous due to the main causative agent of the disease which cannot be cultured in the laboratory. So manipulation of genetic approach is important [17,18]. Gram negative bacteria are characterized by a unique membrane of integral protein that is divided into β-barrel structure consisting of eight to twenty six antiparallel amphipathic strands

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[19,20]. The main function of the β–barrel outer membrane (OMPs) dynamic for membrane biogenesis and transport signaling, enzyme and receptor [21,22]. β-Barrel outer membrane protein is critical aim to conserved domain region and contains N-and C-terminal domains (TprCN and TprCC, respectively) [11].

Currently, the medical research has improved by immunoinformatics technology and usage of such technologies has provided epitopes for creating chimeric, multi-epitope vaccine for several organisms like Hepatitis B Virus or *Escherichia coli* [23]. Proof of identity of B-cell epitopes acting an important role in development of epitope-based vaccines, therapeutic antibodies, and diagnostic tools and T-cell epitopes are shares of intracellular dealing out antigens that are presented to T lymphocytes in association with molecules of the key histocompatibility complex [23,24].

Syphilis vaccination studies are still in the elementary/preclinical stage. The aim of this study is to apply immunoinformatics on β-barrel outer membrane protein to design a safe and effective vaccine. No previous reports were found so this may be considered the first study to use *in silico* approach to design an epitope-based vaccine [25].

MATERIALS AND METHODS

Protein sequence retrieval

A total of 66 *Treponema Pallidum* outer membrane beta-barrel protein strains were retrieved from National Center for Biotechnology Information (NCBI) database on July 2019 in FASTA format. These strains were obtained from different parts of the world for immunoinformatics analysis. The retrieved protein strains had length of 219 amino acid.

Determination of conserved regions

The retrieved sequences of *Treponema pallidum* outer membrane beta-barrel protein were subjected to multiple sequence alignment (MSA) by blasting them against reference sequence (WP_010882178.1) using ClustalW tool of BioEdit Sequence Alignment Editor Software version 7.2-0.5 to determine the conserved regions. Peptides allocated at highly conserved regions will most likely develop in to stronger vaccine that covers more populations. The molecular weight and amino acid composition of the protein were also retrieved [26].

Sequenced-based method

The reference sequence of *Treponema pallidum* outer membrane beta-barrel protein was submitted to different prediction tools at the Immune Epitope Database (IEDB) analysis resource (http://www.iedb.org/) to predict various B and T cell epitopes. Conserved epitopes would be considered as candidate epitopes for B and T cells [27].

B cell epitope prediction

B cell epitopes is the part of the vaccine that interacts with B-lymphocytes. Candidate epitopes were analysed using several B cell prediction methods from IEDB (http://tools.iedb.org/bcell/), to identify the surface accessibility, antigenicity and hydrophilicity with the aid of computerized algorithm. Peptides with length larger than eight peptides were spliced to increase the possibility of obtaining peptides with higher scores in the tests. The Bepipred Linear Epitope Prediction 2 was used to predict linear B cell epitope with default threshold value 0.500 (http://tools.iedb.org/bcell/result/). The Emini Surface Accessibility Prediction tool was used to detect the surface accessibility with default threshold value 1.000 (http://tools.iedb.org/bcell/result/). The kolaskar and tongaonkar antigenicity method was used to identify the antigenicity sites of candidate epitope with default threshold value 1.018 (http://tools.iedb.org/bcell/result/). Parker Hydrophilicity Prediction tool was used to identify the hydrophilic, accessible, or mobile regions with default threshold value 1.447 [28-31].

T cell epitope prediction MHC Class I binding

T cell epitopes is the part of the vaccine that interacts with T lymphocytes. Analysis of peptide binding to the MHC (Major Histocompatibility complex) class I molecule was assessed by the IEDB MHC I prediction tool (http://tools.iedb.org/mhci/). Artificial Neural Network (ANN) 4.0 prediction method was used to predict the binding affinity. Before the prediction, all human allele lengths were selected and set to nine aminoacids. The half-maximal inhibitory concentration (IC50) value required for all conserved epitopes to bind at score less than or equal to 500 were selected [32-34].

T cell epitope prediction MHC Class II binding

Prediction of T cell epitopes interacting with MHC Class II was assessed by the IEDB MHC II prediction tool (http://tools.iedb.org/mhcii/). There are six available tools for prediction: SMM_align, NN-align, Combinatorial Libraries, Sturniolo's method, Net MHCII pan and consensus method. Human allele references set were used to determine the interaction potentials of T cell epitopes and MHC Class II allele (HLA DR, DP and DQ). NN-align method was used to predict the binding affinity. All human allele lengths were set to the standard length. IC50 values at score less than or equal to 100 were selected [35,36].

Population coverage

In IEDB, the population coverage link was selected to analyse the epitopes. This tool calculates the fraction of individuals predicted to respond to a given set of epitopes with known MHC restrictions (http://tools.iedb.org/population/iedbinput). The appropriate checkbox for calculation was checked based on MHC I, MHC II separately and combination of both which is set against the whole world population [37].

AllerTOP v. 2.0

This method is used for allergenicity predictions; it is based on auto cross covariance (ACC) transformation of protein sequences into uniform equal-length vectors. The reference protein sequence (WP_010882178.1) was used. The principal Characteristics of the amino acids were represented by five E descriptors, which indicate amino acid hydrophobicity, molecular size, helix-forming propensity, relative abundance of amino acids, and β-strand forming propensity.

Homology modeling

The 3D structure was obtained using raptorX which is a protein structure prediction server developed by Xu group, excelling at predicting secondary and tertiary structure for protein sequences without close homologs in the Protein Data Bank (PDB). Obtained 3D protein structure was visualized by USCF chimera (version 1.8) which was also used for visualization and analysis of molecular structure of the promising epitopes and it's binding to MHC class I, MHC class II [38,39].

RESULTS

Multiple sequence alignment

The conserved regions and amino acid composition for the

reference sequence of *Treponeman Pallidum* Outer Membrane Beta Barrel Protein are illustrated in Figures 1 and 2 respectively. Glycine, serine and leucine were the most abundant amino acids (Table 1).

B-cell epitope prediction

The reference sequence of *Treponeman Pallidum* Outer Membrane Beta Barrel Protein was subjected to Bepipred linear epitope 2, Emini surface accessibility, Kolaskar and Tongaonkar antigenicity and Parker hydrophilicity prediction methods to test for various immunogenicity parameters (Table 2 and Figures 3-8). Two epitopes have successfully passed the three tests. Three dimensional structure of the proposed B cell epitopes is shown in Figures 7 and 8.

Prediction of cytotoxic T-lymphocyte epitopes and modeling

The reference sequence was analyzed using (IEDB) MHC-1 binding prediction tool to predict T cell epitopes interacting with different types of MHC Class I alleles, based on Artificial Neural Network (ANN) with half-maximal inhibitory concentration (IC50)<100 nm. 36 peptides were predicted to interact with different MHC-

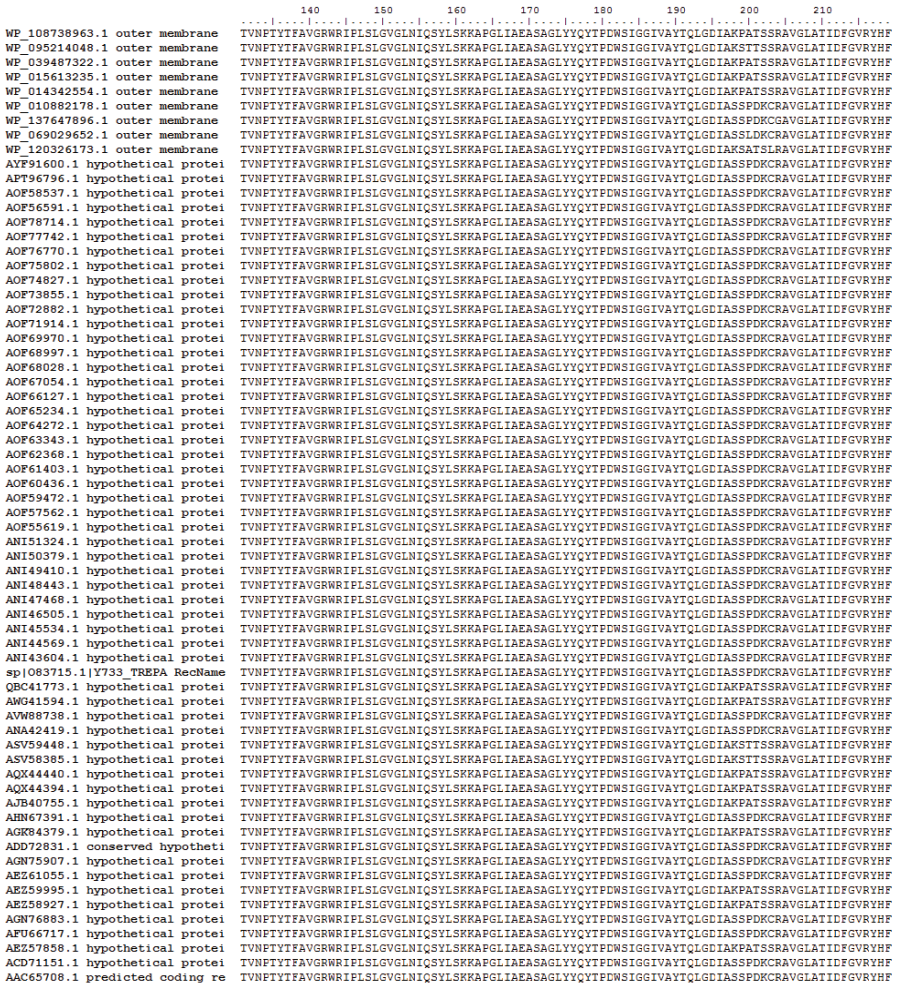

Figure 1: Multiple Sequence Alignment using BioEdit software.

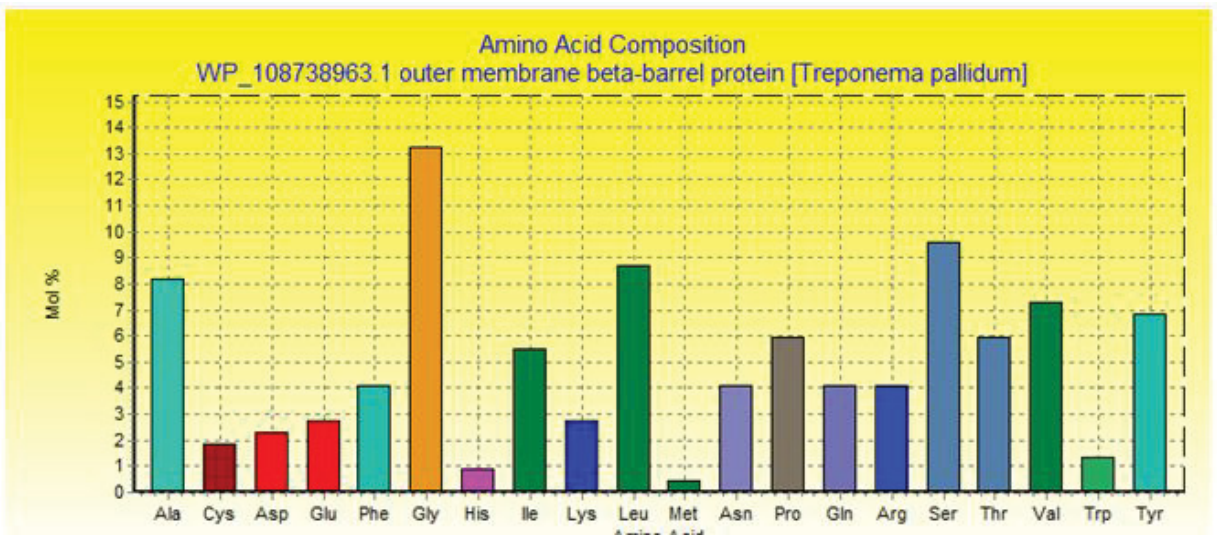

Figure 2: Aminoacid composition for Treponeman Pallidum Outer Membrane Beta Barrel Protein using BioEdit software.

Table 1: Molecular weight and amino acid frequency distribution of the protein.

Amino Acid	Number	Mol%
Ala	18	8.22
Cys	4	1.83
Asp	5	2.28
Glu	6	2.74
Phe	9	4.11
Gly	29	13.24
His	2	0.91
Ile	12	5.48
Lys	6	2.74
Leu	19	8.68
Met	1	0.46
Asn	9	4.11
Pro	13	5.94
Gln	9	4.11
Arg	9	4.11
Ser	21	9.59
Thr	13	5.94
Val	16	7.31
Trp	3	1.37
Tyr	15	6.85

Table 2: List of conserved epitopes that has successfully passed the four tests along with their relative scores. *Proposed epitopes.

Peptide	Start	End	Length	Emini surface accessibility score (TH: 1)	Kolaskar and Tongaonkar antigenicity score (TH: 1.044)	Parker Hydrophilicity prediction score (TH: 1.196)	ALLERTOP
LPAYSSEGVREVPPSQ	21	36	16	3.52	1.058	2.556	Non Allergen
SSEGVREVPPSQSPQV	25	40	16	4.645	1.054	3.669	Non Allergen
EGVREVPPSQSPQVVV	27	42	16	1.425	1.101	2.394	Non Allergen
GQVSFECYRTT	110	120	11	1.091	1.045	2.473	Non Allergen
QVSFECYRTTG	111	121	11	1.091	1.045	2.473	Non Allergen
VSFECYRTTGSNYYF	112	126	15	1.503	1.045	1.447	Non Allergen
FECYRTTGSNYYFSV	114	128	15	1.503	1.045	1.447	Non Allergen
GVGLNIQSYLSKKAPP*	150	165	16	1.101	1.05	1.4	Non Allergen
AEASAGLYYQYTP	168	180	13	2.611	1.05	1.9	Non Allergen
SAGLYYQYTPD*	171	181	11	3.418	1.049	2.064	Non Allergen

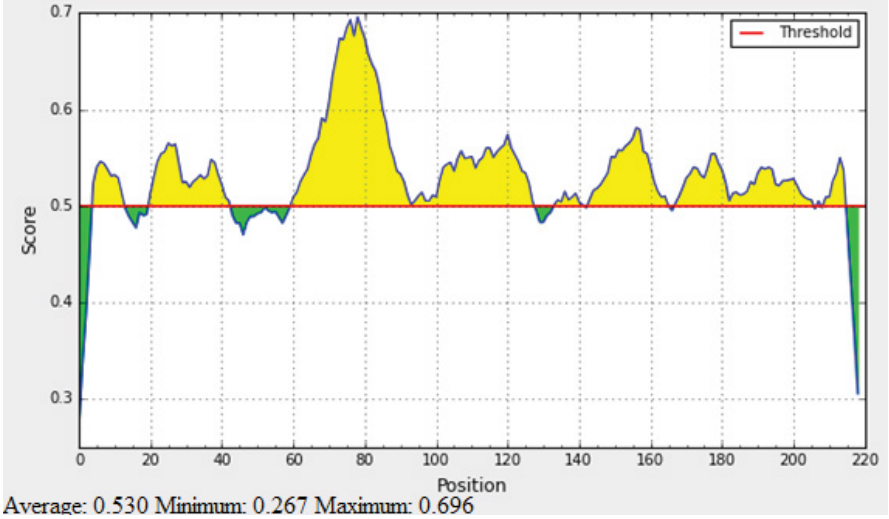

Figure 3: Bepipred Linear Epitope Prediction; Yellow areas above threshold (red line) are proposed to be a part of B cell epitopes and the green areas are not.

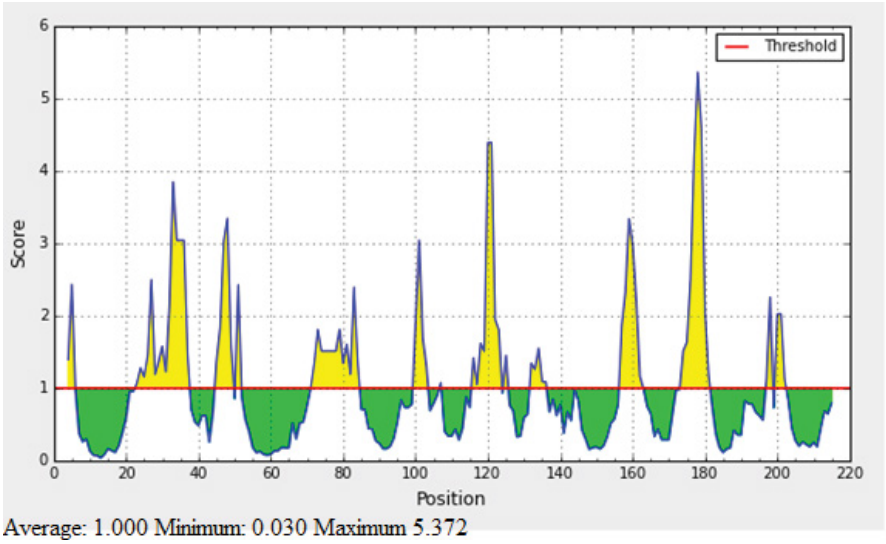

Figure 4: EMINI surface accessibility prediction; Yellow areas above the threshold (red line) are proposed to be a part of B cell epitopes and the green areas are not.

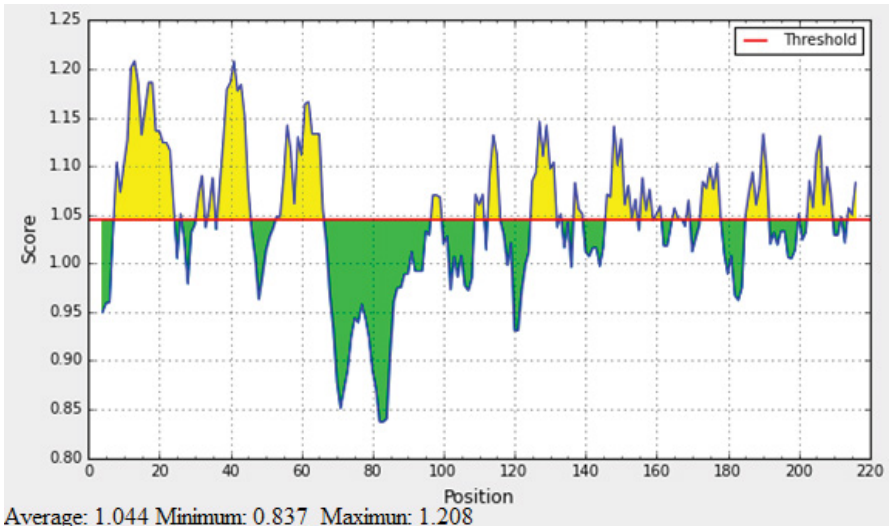

Figure 5: Kolaskar and Tonganokar antigenicity prediction; Yellow areas above the threshold (red line) are proposed to be a part of B cell epitopes and green areas are not.

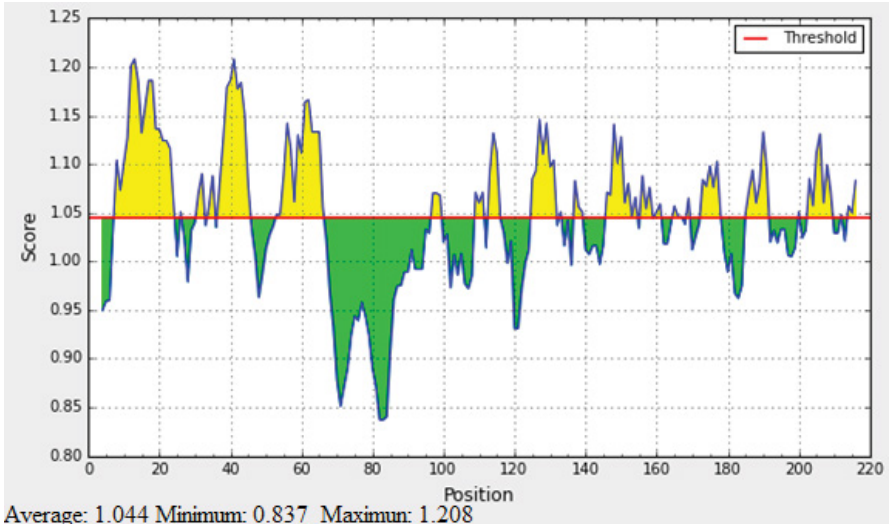

Figure 6: Parker Hydrophilicity prediction; Yellow areas above the threshold (red line) are proposed to be a part of B cell epitopes and green areas are not

1alleles. The most promising epitopes and their corresponding MHC-1 alleles are shown in Table 3. The tertiary structure of the promising epitopes was missing in the homology model.

Prediction of the helper T-lymphocyte epitopes and modeling

Reference sequence was analyzed using (IEDB) MHC-II binding

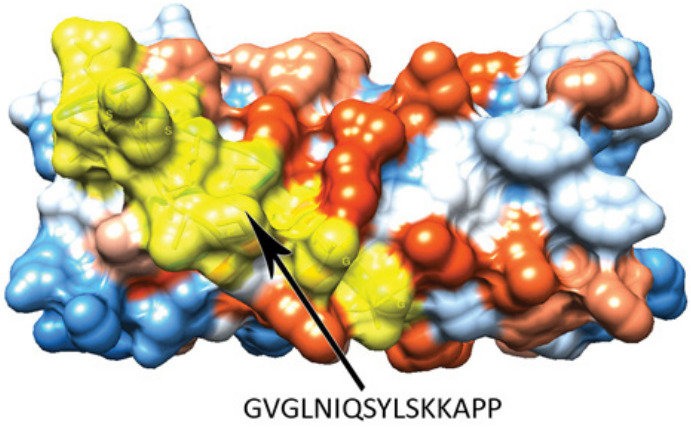

Figure 7: Proposed B cell epitope. The arrow shows position of (GVGLNIQSYLSKKAPP) with yellow colour at structural level using Chimera software.

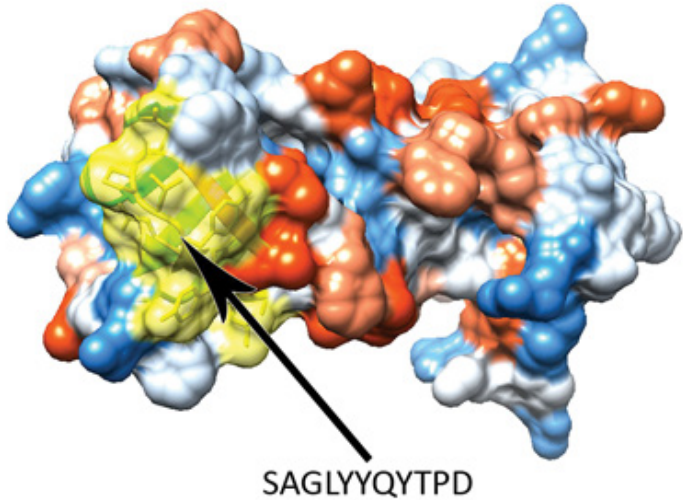

Figure 8: Proposed B cell epitope. The arrow shows position of (SAGLYYQYTPD) with yellow colour at structural level using Chimera software.

Table 3: The most promising T cell epitopes and their corresponding MHC-1 alleles.

Peptide	MHC 1 alleles
AEASAGLYY	HLA-B*44:03, HLA-B*44:02, HLA-B*15:01, HLA-A*29:02
ALCALSPLL	HLA-A*02:01
ALSPLLPAY	HLA-B*15:01, HLA-A*30:02, HLA-A*29:02
ASAGLYYQY	HLA-A*29:02, HLA-A*30:02, HLA-B*58:01, HLA-A*11:01
CALSPLLPA	HLA-A*02:06
CYRTTGSNY	HLA-C*14:02
FSLGGQVSF	HLA-C*03:03, HLA-B*35:01
GALCALSPL	HLA-A*02:06
GQVSFECYR	HLA-A*31:01
GRWRIPLSL	HLA-B*27:05
IAEASAGLY	HLA-A*30:02
IPLSLGVGL	HLA-B*07:02
ITVNPTYTF	HLA-A*32:01, HLA-B*58:01, HLA-B*57:01, HLA-A*23:01
LGYEYFFTK	HLA-A*11:01
LIAEASAGL	HLA-A*02:06, HLA-A*68:02
LYYQYTPDW	HLA-C*14:02, HLA-A*23:01, HLA-A*24:02
MSRTFRAWQ	HLA-A*30:01
NIQSYLSKK	HLA-A*11:01, HLA-A*68:01
QCVGALCAL	HLA-C*03:03
QVVVAYEPI	HLA-A*68:02, HLA-A*02:06
RAWQCVGAL	HLA-B*07:02, HLA-C*03:03, HLA-C*12:03
RTFRAWQCV	HLA-A*30:01, HLA-A*02:06, HLA-A*32:01, HLA-C*15:02, HLA-C*12:03
RTTGSNYYF	HLA-B*58:01, HLA-B*57:01, HLA-A*32:01
SNYYFSVPI	HLA-A*32:01, HLA-A*68:02
TVNPTYTFA	HLA-A*68:02
VAYEPIRPG	HLA-C*12:03
VPITVNPTY	HLA-B*35:01, HLA-B*53:01
WRIPLSLGV	HLA-B*27:05
WSIGGIVAY	HLA-B*35:01, HLA-A*29:02, HLA-C*12:03, HLA-B*15:01
YEYFFTKNF	HLA-B*18:01, HLA-C*12:03, HLA-B*44:03, HLA-B*40:02, HLA-C*14:02, HLA-B*44:02
YFFTKNFSL	HLA-C*14:02, HLA-C*03:03, HLA-B*08:01
YLSKKAPGL	HLA-A*02:01
YQYTPDWSI	HLA-A*02:06, HLA-A*02:01, HLA-C*03:03, HLA-B*39:01, HLA-C*12:03
YRTTGSNYY	HLA-C*06:02, HLA-C*07:01
YSSEGVREV	HLA-C*12:03
YYFSVPITV	HLA-C*12:03, HLA-C*14:02, HLA-A*23:01, HLA-A*24:02, HLA-C*07:02

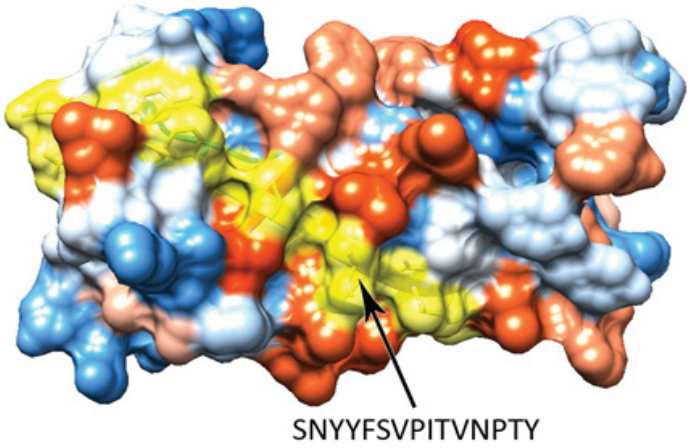

Figure 9: Proposed T cell epitopes that interact with MHC2.The arrow shows position of (SNYYFSVPITVNPTY) with yellow colour at structural level using Chimera software.

prediction tool based on NN-align with half-maximal inhibitory concentration (IC50)<500 nm; there were 135 predicted epitopes found to interact with MHC-II alleles. The most promising epitopes and their corresponding alleles are shown in Table 4 along with the 3D structure of the proposed epitope (Figure 9).

Population coverage analysis

All MHC I and MHC II epitopes were assessed for population coverage against the whole world using IEDB population coverage tool. For MHC 1, epitopes with highest population coverage were YQYTPDWSI (52.63) and YYFSVPITV (50.62) (Figure 10 and Ta-

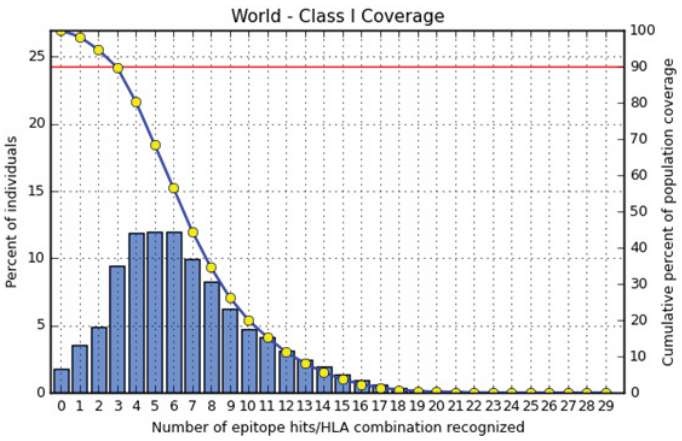

Figure 10: Population coverage for MHC class I epitopes.

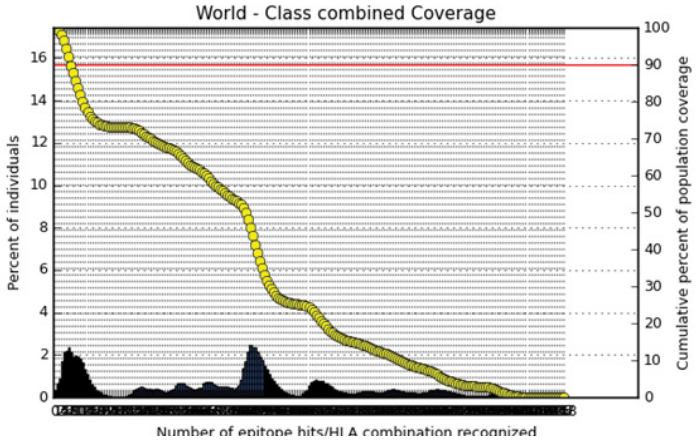

Figure 11: Population coverage for combined MHC I and II epitopes.

Table 4: The most promising T cell epitopes and their corresponding MHC-2 alleles.

Peptide	MHC 2 alleles
SNYYFSVPITVNPTY	HLA-DRB1*09:01, HLA-DRB1*11:01, HLA-DRB1*04:01, HLA-DPA1*03:01/DPB1*04:02, HLA-DRB1*15:01, HLA-DPA1*02:01/DPB1*01:01, HLA-DRB1*04:05, HLA-DRB1*04:04, HLA-DRB1*13:02, HLA-DRB1*01:01, HLA-DQA1*05:01/DQB1*03:01, HLA-DRB1*07:01, HLA-DQA1*01:01/DQB1*05:01, HLA- DQA1*01:02/DQB1*06:02, HLA-DRB1*08:02, HLA-DPA1*01/DPB1*04:01 QVVVAYEPIRPGDQL HLA-DRB1*15:01, HLA-DRB4*01:01, HLA-DRB1*01:01, HLA-DRB1*08:02, HLA-DRB1*04:04, HLA-DPA1*03:01/DPB1*04:02, HLA-DQA1*05:01/DQB1*02:01
GSNYYFSVPITVNPT	HLA-DRB1*09:01, HLA-DRB1*11:01, HLA-DRB1*15:01, HLA-DRB1*04:01, HLA-DPA1*03:01/DPB1*04:02, HLA-DRB1*04:05, HLA-DPA1*02:01/DPB1*01:01, HLA-DRB1*13:02, HLA-DRB1*04:04, HLA-DRB1*01:01, HLA-DQA1*05:01/DQB1*03:01, HLA-DRB1*07:01, HLA-DQA1*01:01/DQB1*05:01, HLA-DQA1*01:02/DQB1*06:02, HLA-DRB5*01:01, HLA-DPA1*01/DPB1*04:01 SIGGIVAYTQLGDIA HLA-DPA1*02:01/DPB1*01:01, HLA-DPA1*01:03/DPB1*02:01, HLA-DRB1*15:01, HLA-DRB1*07:01, HLA-DRB1*04:04, HLA-DQA1*05:01/DQB1*03:01, HLA-DRB1*01:01, HLA-DRB4*01:01, HLA-DRB1*04:05, HLA-DPA1*03:01/DPB1*04:02, HLA-DRB1*08:02
EYFFTKNFSLGGQVS	HLA-DRB5*01:01, HLA-DPA1*01:03/DPB1*02:01, HLA-DRB1*09:01, HLA-DPA1*02:01/DPB1*01:01, HLA-DRB1*04:05, HLA-DRB1*15:01, HLA-DPA1*03:01/DPB1*04:02, HLA-DRB1*01:01, HLA-DRB1*11:01, HLA-DRB1*07:01, HLA-DRB1*04:01, HLA-DPA1*01/DPB1*04:01, HLA-DPA1*02:01/DPB1*05:01, HLA-DQA1*05:01/DQB1*03:01, HLA-DRB1*13:02, HLA-DRB1*04:04
YFFTKNFSLGGQVSF	HLA-DRB1*09:01, HLA-DRB1*04:01, HLA-DPA1*01/DPB1*04:01, HLA-DRB5*01:01, HLA-DQA1*05:01/DQB1*03:01, HLA-DPA1*01:03/DPB1*02:01, HLA-DPA1*02:01/DPB1*01:01, HLA-DRB1*04:05, HLA-DRB1*01:01, HLA-DRB1*11:01, HLA-DRB1*07:01, HLA-DRB1*15:01, HLA-DPA1*03:01/DPB1*04:02, HLA-DRB1*04:04, HLA-DRB1*13:02
LGYEYFFTKNFSLGG	HLA-DRB1*04:05, HLA-DPA1*02:01/DPB1*05:01, HLA-DRB1*15:01, HLA-DRB1*04:04, HLA-DRB1*09:01, HLA-DPA1*03:01/DPB1*04:02, HLA-DRB1*01:01, HLA-DRB1*07:01, HLA-DPA1*01/DPB1*04:01, HLA-DRB5*01:01, HLA-DPA1*02:01/DPB1*01:01, HLA-DPA1*01:03/DPB1*02:01, HLA-DRB1*04:01, HLA-DRB1*11:01, HLA-DQA1*01:01/DQB1*05:01

Table 5: Population coverage of proposed peptides interaction with MHC class I.

Epitope	Coverage Class 1 (%)	Total HLA hits
YQYTPDWSI	0.5263	5
YYFSVPITV	0.5062	5
ALCALSPLL	0.3908	1
YLSKKAPGL	0.3908	1
YEYFFTKNF	0.3359	6
YRTTGSNYY	0.3331	2
RAWQCVGAL	0.2847	3
LYYQYTPDW	0.2843	3
WSIGGIVAY	0.2801	4
AEASAGLYY	0.2452	4
ASAGLYYQY	0.2397	4
RTFRAWQCV	0.2327	5
NIQSYLSKK	0.2088	2
YFFTKNFSL	0.2042	3
ITVNPTYTF	0.1645	4
FSLGGQVSF	0.1585	2
LGYEYFFTK	0.1553	1
ALSPLLPAY	0.1418	3
IPLSLGVGL	0.1278	1
RTTGSNYYF	0.1153	3
VPITVNPTY	0.1084	2
VAYEPIRPG	0.1031	1
YSSEGVREV	0.1031	1
QCVGALCAL	0.0812	1
SNYYFSVPI	0.0705	2
GQVSFECYR	0.0536	1
GRWRIPLSL	0.0478	1
WRIPLSLGV	0.0478	1
LIAEASAGL	0.0443	2
QVVVAYEPI	0.0443	2

ble 5). For MHC class II, the epitopes that showed highest population coverage were SNYYFSVPITVNPTY (73.11), GSNYYFSVPIT-

Table 6: Population coverage of proposed peptides interaction with MHC class 2.

Epitope	Coverage Class 2 (%)	Total HLA hits
SNYYFSVPITVNPTY	0.7311	16
GSNYYFSVPITVNPT	0.7188	16
EYFFTKNFSLGGQVS	0.7188	16
YFFTKNFSLGGQVSF	0.7188	15
YEYFFTKNFSLGGQV	0.7188	15
TGSNYYFSVPITVNP	0.7188	14
IPLSLGVGLNIQSYL	0.7024	11
TTGSNYYFSVPITVN	0.6926	13
LNIQSYLSKKAPGLI	0.6887	10
LGYEYFFTKNFSLGG	0.6815	15
GYEYFFTKNFSLGGQ	0.6815	14
PLSLGVGLNIQSYLS	0.6776	11
LSLGVGLNIQSYLSK	0.6776	11
ITVNPTYTFAVGRWR	0.6755	10
NIQSYLSKKAPGLIA	0.6755	9
IQSYLSKKAPGLIAE	0.6755	9
GLNIQSYLSKKAPGL	0.6496	9
HLGYEYFFTKNFSLG	0.6437	14
RIPLSLGVGLNIQSY	0.6404	10
FFTKNFSLGGQVSFE	0.6356	8
FTKNFSLGGQVSFEC	0.6356	8
YFSVPITVNPTYTFA	0.6298	10
RTTGSNYYFSVPITV	0.6296	9
NYYFSVPITVNPTYT	0.6214	13
YYFSVPITVNPTYTF	0.6214	13
SAGLYYQYTPDWSIG	0.6174	15
AGLYYQYTPDWSIGG	0.6174	14
LYYQYTPDWSIGGIV	0.6174	14
ASAGLYYQYTPDWSI	0.6174	14
GLYYQYTPDWSIGGI	0.6174	13
QVSFECYRTTGSNYY	0.6174	10
VSFECYRTTGSNYYF	0.6174	9
SFECYRTTGSNYYFS	0.6174	9
FECYRTTGSNYYFSV	0.6174	9
FSVPITVNPTYTFAV	0.6111	9
SVPITVNPTYTFAVG	0.6111	8
PGLIAEASAGLYYQY	0.6109	10
APGLIAEASAGLYYQ	0.6109	10
YTFAVGRWRIPLSLG	0.6086	13
TFAVGRWRIPLSLGV	0.6086	12
VGRWRIPLSLGVGLN	0.6086	9
FAVGRWRIPLSLGVG	0.6086	8
AVGRWRIPLSLGVGL	0.6086	8
QSYLSKKAPGLIAEA	0.6064	9
WRIPLSLGVGLNIQS	0.5984	7
TYTFAVGRWRIPLSL	0.5939	12
GLIAEASAGLYYQYT	0.5672	10
KAPGLIAEASAGLYY	0.5672	8
KKAPGLIAEASAGLY	0.5672	8
SKKAPGLIAEASAGL	0.5672	7
NPTYTFAVGRWRIPL	0.5625	9
VNPTYTFAVGRWRIP	0.5625	9
TVNPTYTFAVGRWRI	0.5625	8
GRWRIPLSLGVGLNI	0.5625	7
LGVGLNIQSYLSKKA	0.5597	11
PTYTFAVGRWRIPLS	0.5513	10
VGLNIQSYLSKKAPG	0.5271	8
GVGLNIQSYLSKKAP	0.5271	8
SLGVGLNIQSYLSKK	0.5154	10
SIGGIVAYTQLGDIA	0.5141	11
WSIGGIVAYTQLGDI	0.5141	11
GALCALSPLLPAYSS	0.5064	13
VGALCALSPLLPAYS	0.5064	13
ALCALSPLLPAYSSE	0.5064	12
LCALSPLLPAYSSEG	0.5014	10
QCVGALCALSPLLPA	0.4899	12
WQCVGALCALSPLLP	0.4884	11
YYQYTPDWSIGGIVA	0.4884	9
LIAEASAGLYYQYTP	0.4879	9
RWRIPLSLGVGLNIQ	0.4879	6
SYLSKKAPGLIAEAS	0.4757	8
CVGALCALSPLLPAY	0.4679	11
CYRTTGSNYYFSVPI	0.4628	7
YRTTGSNYYFSVPIT	0.4628	7
MSRTFRAWQCVGALC	0.4627	6
FRAWQCVGALCALSP	0.4612	8
RTFRAWQCVGALCAL	0.4612	7
TFRAWQCVGALCALS	0.4612	7
SRTFRAWQCVGALCA	0.4612	7
SPQVVVAYEPIRPGD	0.4527	10
VPITVNPTYTFAVGR	0.4504	6
PQVVVAYEPIRPGDQ	0.4478	10
ECYRTTGSNYYFSVP	0.4403	5
YQYTPDWSIGGIVAY	0.4306	7
CALSPLLPAYSSEGV	0.422	8
RAWQCVGALCALSPL	0.4124	7
AWQCVGALCALSPLL	0.4124	7
ALSPLLPAYSSEGVR	0.404	7
YLSKKAPGLIAEASA	0.3943	7
LSPLLPAYSSEGVRE	0.3803	5
PITVNPTYTFAVGRW	0.3615	5
DWSIGGIVAYTQLGD	0.3527	7
EASAGLYYQYTPDWS	0.35	7
GQVSFECYRTTGSNY	0.3484	6
QVVVAYEPIRPGDQL	0.3471	7
VVVAYEPIRPGDQLL	0.3471	4
AVGLATIDFGVRYHF	0.3444	3
TKNFSLGGQVSFECY	0.3402	5
KNFSLGGQVSFECYR	0.3402	5
QSPQVVVAYEPIRPG	0.3055	8
SQSPQVVVAYEPIRP	0.2858	5
SPLLPAYSSEGVREV	0.2187	5
PIRPGDQLLKIGIVA	0.2143	3
IRPGDQLLKIGIVAG	0.2143	2
YEPIRPGDQLLKIGI	0.1784	1

LSKKAPGLIAEASAG	0.1782	4
TPDWSIGGIVAYTQL	0.1755	5
NFSLGGQVSFECYRT	0.1755	5
YTPDWSIGGIVAYTQ	0.1755	4
QYTPDWSIGGIVAYT	0.1755	4
LGGQVSFECYRTTGS	0.157	4
GGQVSFECYRTTGSN	0.157	4
PDWSIGGIVAYTQLG	0.1153	3
AYEPIRPGDQLLKIG	0.1153	1
VAYEPIRPGDQLLKI	0.1153	1
VVAYEPIRPGDQLLK	0.1153	1
AEASAGLYYQYTPDW	0.0771	4
FSLGGQVSFECYRTT	0.064	5
IAEASAGLYYQYTPD	0	4
SLGGQVSFECYRTTG	0	2
PLLPAYSSEGVREVP	0	2
LPAYSSEGVREVPPS	0	2
VPPSQSPQVVVAYEP	0	2
PPSQSPQVVVAYEPI	0	2
LLPAYSSEGVREVPP	0	2
SEGVREVPPSQSPQV	0	1
PAYSSEGVREVPPSQ	0	1
PSQSPQVVVAYEPIR	0	1
GVREVPPSQSPQVVV	0	1
EPIRPGDQLLKIGIV	0	1
SSEGVREVPPSQSPQ	0	1
EGVREVPPSQSPQVV	0	1
YSSEGVREVPPSQSP	0	1
AYSSEGVREVPPSQS	0	1
VREVPPSQSPQVVVA	0	1

Table 7: Population coverage of proposed peptide interaction with MHC class 1 and 2 combined.

Epitope	Coverage Class 1 and 2 combined (%)	Total HLA hits
SNYYFSVPITVNPTY	0.7311	16
GSNYYFSVPITVNPT	0.7188	16
EYFFTKNFSLGGQVS	0.7188	16
YFFTKNFSLGGQVSF	0.7188	15
YEYFFTKNFSLGGQV	0.7188	15
TGSNYYFSVPITVNP	0.7188	14
IPLSLGVGLNIQSYL	0.7024	11
TTGSNYYFSVPITVN	0.6926	13
LNIQSYLSKKAPGLI	0.6887	10
LGYEYFFTKNFSLGG	0.6815	15
GYEYFFTKNFSLGGQ	0.6815	14
PLSLGVGLNIQSYLS	0.6776	11
LSLGVGLNIQSYLSK	0.6776	11
ITVNPTYTFAVGRWR	0.6755	10
NIQSYLSKKAPGLIA	0.6755	9
IQSYLSKKAPGLIAE	0.6755	9
GLNIQSYLSKKAPGL	0.6496	9
HLGYEYFFTKNFSLG	0.6437	14
RIPLSLGVGLNIQSY	0.6404	10
FFTKNFSLGGQVSFE	0.6356	8
FTKNFSLGGQVSFEC	0.6356	8
YFSVPITVNPTYTFA	0.6298	10
RTTGSNYYFSVPITV	0.6296	9
NYYFSVPITVNPTYT	0.6214	13
YYFSVPITVNPTYTF	0.6214	13
SAGLYYQYTPDWSIG	0.6174	15
AGLYYQYTPDWSIGG	0.6174	14
LYYQYTPDWSIGGIV	0.6174	14
ASAGLYYQYTPDWSI	0.6174	14
GLYYQYTPDWSIGGI	0.6174	13
QVSFECYRTTGSNYY	0.6174	10
VSFECYRTTGSNYYF	0.6174	9
SFECYRTTGSNYYFS	0.6174	9
FECYRTTGSNYYFSV	0.6174	9
FSVPITVNPTYTFAV	0.6111	9
SVPITVNPTYTFAVG	0.6111	8
PGLIAEASAGLYYQY	0.6109	10
APGLIAEASAGLYYQ	0.6109	10
YTFAVGRWRIPLSLG	0.6086	13
TFAVGRWRIPLSLGV	0.6086	12
VGRWRIPLSLGVGLN	0.6086	9
FAVGRWRIPLSLGVG	0.6086	8
AVGRWRIPLSLGVGL	0.6086	8
QSYLSKKAPGLIAEA	0.6064	9
WRIPLSLGVGLNIQS	0.5984	7
TYTFAVGRWRIPLSL	0.5939	12
GLIAEASAGLYYQYT	0.5672	10

Table 8: The population coverage of whole world for the epitope set for MHC I, MHC II and MHC I and II combined.

Country	MHC I	MHC II	MHC I and II combined
World	98.2%	68.45% *	99.64% *

Table 9: Population coverage for MHC class II epitopes.

Population/Area	Class II		
World	Coverage[a]	Average_hit[b]	pc90[c]
Average	68.45%	53.07	-1.16
Standard Deviation	0.0	0.0	0.0

VNPT and EYFFTKNFSLGGQVS (71.88%) (Tables 6-9). When combined together, the epitopes that showed highest population coverage were SNYYFSVPITVNPTY (73.11), GSNYYFSVPITVNPT and EYFFTKNFSLGGQVS (71.88%) (Figure 11, Tables 7 and 8).

In population coverage analysis of MHC II; 14 alleles were not included in the calculation, therefore the above (*) percentages are for epitope sets excluding: HLA-DQA1*05:01/DQB1*03:01, HLA-DQA1*01:02/DQB1*06:02, HLA-DPA1*01:03/DPB1*02:01, HLA-DPA1*01/DPB1*04:01, HLA-DPA1*02:01 /DPB1*05:01, HLA-DQA1*01:01/DQB1*05:01, HLA-DQA1*03:01/DQB1*03:02, HLA-DRB3*01:01, HLA-DRB4*01:01, HLA-DRB5*01:01, HLA-DQA1*04:01/DQB1*04:02, HLA-DPA1*03:01/DPB1*04:02, HLA-DQA1*05:01/DQB1*02:01, HLA-DPA1*02:01/DPB1*01:01.

DISCUSSION

In this study, we successfully designed a peptide vaccine for *Treponema*

pallidum against its immunogenic outer membrane beta-barrel protein by immunoinformatics tools. These peptides can be recognized by B cell and T cell to produce antibodies. Predicted peptide vaccine characterized by easy production, stimulating effective immune response and no potential infection possibilities. Peptide vaccines overcome the side effects of conventional vaccines [40].

The reference sequence of *Treponema pallidum* outer membrane beta-barrel protein was subjected to Bepipred linear epitope prediction 2 test, Emini surface accessibility test, Kolaskar and Tongaonkar antigenicity test and Parker hydrophilicity test in IEDB, to determine the binding to B cell, surface accessibility, antigenicity, the hydrophilicity of the B cell epitope respectively. Out of the ten predicted epitopes using Bepipred 2 test, only two epitopes passed the other three tests (GVGLNIQSYLSKKAPP, SAGLYYQYTPD). The reference sequence was analysed using MHC Iand MHC II binding prediction tools in IEDB to predict T cell epitopes. 36 epitopes were predicted to interact with MHC I alleles with IC50<100. Four of them were most promising and had the affinity to bind the highest number of MHC1 alleles (YEYFFTKNF, YQYTPDWSI, YYFSVPITV, RTFRAWQCV). 135 epitopes predicted to interact with MHC II alleles with IC50<500. Five of them were most promising and had the affinity to bind to the highest number of MHC II alleles (SNYYFSVPITVNPTY, GSNYYFSVPITVNPT, EYFFTKNFSLGGQVS, YFFTKNFSLGGQVSF, LGYEYFFTKNFSLGG).

The best epitope with the highest population coverage for MHC I was YQYTPDWSI with 52.63% in five HLA hits, and the coverage of population set for whole MHC I epitopes was %. Excluding certain alleles for MHC II, the best epitope was SNYYFSVPITVNPTY scoring 73.11% with 16 HLA hits and the coverage of population set was 68.45% for the whole Helper T-lymphocyte epitopes (MHC II). In population coverage analysis of MHC II; 14 alleles were not included in the calculation: HLA-DQA1*05:01/DQB1*03:01, HLA-DQA1*01:02/DQB1*06:02, HLA-DPA1*01:03/DPB1*02:01, HLA-DPA1*01/DPB1*04:01,HLA-DPA1*02:01/DPB1*05:01,HLA-DQA1*01:01/DQB1*05:01, HLA-DQA1*03:01/DQB1*03:02, HLA-DRB3*01:01, HLA-DRB4*01:01, HLA-DRB5*01:01, HLA-DQA1*04:01/DQB1*04:02,HLA-DPA1*03:01/DPB1*04:02, HLA-DQA1*05:01/DQB1*02:01, HLA-DPA1*02:01/DPB1*01:01. These epitopes could potentially induce a T-cell immune response when interacting strongly with MHC I and MHC II alleles effectively generating a cellular immune response against the invading pathogen. The peptide SNYYFSVPITVNPTY had the highest population coverage per cent 73.1% in 16 HLA hits for both MHC I and MHC II. Many studies have used immunoinformatics methods to predict peptide vaccines for various micro-organisms such as HPV, Rubella, Dengue, Zika, Ebola, and mycetoma [41-49]. We hope that the whole world will benefit from this epitope-based vaccine upon its successful development following *in vivo* and *in vitro* studies to prove its effectiveness.

CONCLUSION

The method to protect and minimize the chance of infection is Vaccination. Vaccines Design by *in silico* prediction methods is very appreciated due to the important reduction in cost, effort and time. Peptide vaccines overcome the side effects of conventional vaccines. For the first time we existing different peptides that can create antibodies against β-Barrel outer membrane protein of *Treponema pallidum*. Two B cell epitopes passed the antigenicity, accessibility and hydrophilicity tests. 36 MHC I epitopes were the most promising ones, while five epitopes for MHC II. Five epitopes were common between MHC I and II. For the population coverage, the epitopes covered 71.88% of the alleles worldwide without certain alleles.

DATA AVAILABILITY

The data which support our findings in this study are available from the corresponding author upon reasonable request.

COMPETING INTEREST

The authors declare that they have no competing interests.

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