Translationally controlled tumor protein *TCTP* as Peptide Vaccine against *Schistosoma japonicum*: an immunoinformatics approach.

Rayan A Abdalrahman[1,2], Shima S Ahmed[2], Mahmoud A Elnaeem[2], Marwa S Mohammed[2], Nawraz M Jammie[2], Israa A Yousif [3,2], Wala H Mohamed[2], Sabreen Y Nasr[2], Mawadda A Awad-Elkareem[2] and Mohamed A Hassan[2]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Khartoum- Khartoum, Sudan

[2]Department of Biotechnology, Africa City of Technology- Khartoum, Sudan

[3]Department of Chemical Pathology, Faculty of Medical Laboratory Sciences, University of Khartoum- Khartoum, Sudan

Abstract

Schistosoma japonicum is the most pathogenic causative form of schistosomiasis that causes a major health problem in its endemic countries. Until now, praziquantel is the only drug used to treat Schistosomiasis, but it does not prevent re-infection. So, repetition of the treatment is needed. Moreover, there is no effective vaccine against S. japonicum. Therefore, an urgent need for the development of vaccines is mandatory. This study aimed to analyze an immunogenic protein, Transitionally Controlled Tumor Protein (TCTP) using an immunoinformatics approach to design a universal peptide vaccine against Schistosoma japonicum. A set of 22 of TCTP sequences were retrieved from NCBI database. Conservancy of these sequences was tested and then conserved B cell and T cell epitopes were predicted using different tools available in Immune Epitope Database IEBD. Epitopes having high scores in both B and T cell predicting tools were proposed. An epitope ^{129}YEHYI^{133} was predicted as a most promising epitope with good prediction scores in surface accessibility and antigenicity. Besides that, epitopes ^{129}YEHYIGESM ^{137} and ^{92}YLKAIKERL^{100}

were predicted as the most promising epitopes concerning their binding to MHC I and MHC II allele respectively. The study revealed that our predicted epitopes could be used to develop an efficacious vaccine against Schistosoma japonicum in the future especially epitope YEHYIGESM as it is shared between MHC I and II alleles and overlapped with the most promising B cell epitope. Both in vitro and in vivo studies is recommended to confirm the efficacy of YEHYIGESM as a peptide vaccine.

Keywords: *Schistosoma japonicum*, *TCTP*, Peptide vaccine, Immune Epitope Database (IEDB), and Immunoinformatics.

Introduction

Schistosoma japonicum is blood flukes that can infect human and livestock causing a chronic disease of considerably importance in public health in endemic countries (1-3). It belongs to the Schistosoma genus of the Schistosomatidae family, which includes mammalian Schistosoma such as *S. jabonicum*, *S. heamatobium*, *S. mansoni, S. intercalatum, and S. mekongi*. This parasite infects human through skin from fresh water infected by larval of parasite that released from snail and lives in the blood vessels causing immune reaction and progressive damage to organs (4-6). Among all species, *Schistosomiasis japonica* is the most pathogenic type since it releases high number of eggs by adult female worm, which deposited in liver, intestine and other organs and induce reaction of parasite with organ (7, 8). Currently, schistosomiasis is endemic in around 74 countries, and more than 200 million people infected by its worldwide (4, 6, 9-12). Schistosomiasis japonica is still endemic in China, Philippines and to some extent Indonesia despite many controlling programs conducted (13, 14).

Till date, praziquantel (PZQ) is the only drug that has been used to treat Schistosomiasis since 1970, but it does not prevent re-infection. So, repetition of the treatment with PZQ is required at frequent intervals (15-19). Additionally, poor activity of praziquantel against immature schistosome, its limited use for patients developed hepatosplenic lesion, and the potential of drug

resistance development prohibit influential control depending on chemotherapy alone and necessitate develop and use of vaccines as a key and complementary component beside other integrated approach for the disease control and elimination (4, 9, 15, 19-24).

In previous studies, the use of recombinant proteins such as, Paramyosin (sj97), Thyroid hormone receptor beta (*SjTHRβ*) induced 33–34%, 27% and 33% reductions of the worm burden in vaccinated animals respectively (25-28), while DNA vaccines like *Sj26GST* induced 30% reductions of the worm burden and 45% reduction of the liver egg burden in vaccinated mice (28, 29). Still, no vaccine was able to induce good protection, thus searching for additional vaccine candidates is needed (21, 28, 30). *Schistosoma japonicum* ***TCTP*** has been identified as a potential target for vaccine design. According to proteomic analysis, Translationally Controlled Tumor Protein (*TCTP*) has been identified in Schistosomulum adult life cycle stage of *S. japonicum* and *S. mansoni*, the stage where the parasite is found in circulatory system and release eggs. It is a highly conserved protein responsible for immunogenic response inside the host body through its mechanism such as histamine releasing and regulation of B cell reactivity (4, 9). Furthermore, a significant protection against *Schistosoma japonicum* was obtained after inoculating mice with TCTP and adjuvant (CpG) (31). So, *Schistosoma japonicum TCTP* was considered a good target for vaccination. Therefore, the aim of this study is to predict effective B and T cell epitopes from *S. japonicum TCTP* protein by means of an immunoinformatics approach.

Recently, immunoinformatics approach has been accepted as a universal tool in the field of vaccine development. This method can help in the prediction of appropriate epitopes for designing an efficacious epitope-based peptide vaccine, and offers high degree of confidence for the prediction of epitopes, as an epitope selection is a critical step in the design of an epitope-based peptide vaccine (32-37). Our present study is the first study using in silico approach to predict all possible B and T cell epitopes from *Schistosoma japonicum TCTP* protein.

Materials and Methods

An outline of the methodology used in this study has been represented in Figure 1.

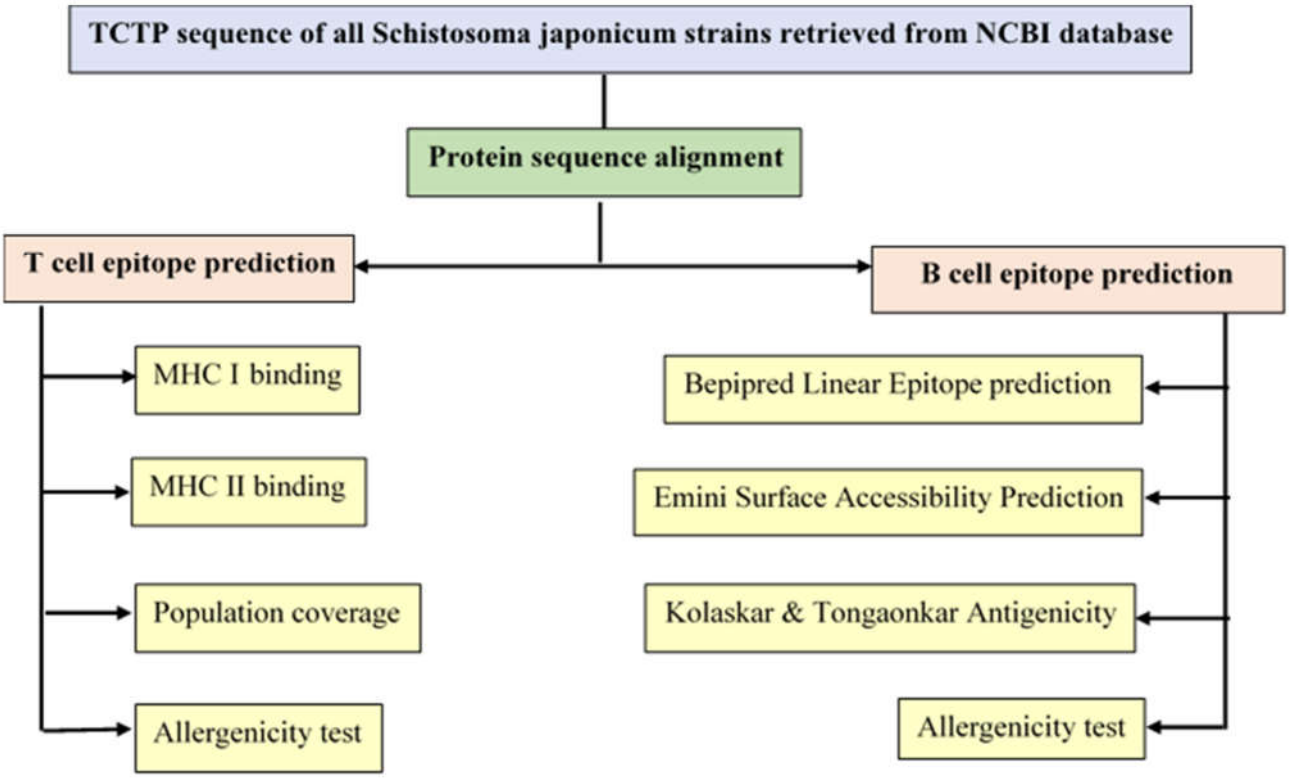

Figure 1. Flowchart representing an immunoinformatics approach used in the prediction of potential B and T cell epitopes in order to develop peptide vaccine against *Schistosoma japonicum*.

Protein sequence retrieval:

A series of 22 sequences of *Schistosoma japonicum* translationally-controlled tumor protein (*TCTP*) were retrieved from the National Center for Biotechnology Information database (NCBI) (**http://www.ncbi.nlm.nih.gov/protein/**) in 9th of December 2017 for immunoinformatic analysis. The retrieved protein sequences with a length of 169 aa were collected from China; retrieved strains and their accession numbers besides collection area are listed in **Table 1**. Protein sequence (**Accession NO CAX83040.1**) was excluded during retrieval from NCBI database, as it has a length of 196 aa instead of 169 aa.

Table 1. Parasite Strains retrieved with their Accession numbers and area of collection.

Accession number	Country
P91800.1*	N.A
AAB42079.1	N.A
CAX82694.1	China
CAX77596.1	China
CAX77595.1	China
CAX77594.1	China
CAX77593.1	China
CAX77592.1	China
CAX77591.1	China
CAX77590.1	China
CAX77589.1	China
CAX77588.1	China
CAX77587.1	China
CAX77586.1	China
CAX77585.1	China
CAX71620.1	China
CAX71619.1	China
CAX71618.1	China
CAX71617.1	China
CAX71616.1	China
CAX71615.1	China
ACE06853.1	N.A

N.A: not available. **P91800 .1*** the protein sequence used in all immuninformatics analysis processes and 3D structure prediction.

Phylogenetic analysis of the protein sequences:

The retrieved sequences were subjected to phylogenetic analysis so as to determine the origin of each strain using Phylogeny.fr software (**http://www.phylogeny.fr**) (38).

Protein sequences conservancy analysis:

The retrieved sequences were aligned using BioEdit sequence alignment editor (v7.2.5) to get the conserved regions with ClustalW as implemented in the BioEdit program (39). Only conserved regions of the protein sequences were selected for immunoinformatics analysis.

B cell epitopes prediction:

As a result of interaction between the B-cell epitopes and the B-lymphocyte, the latter is differentiated into antibody-secreting plasma cell and memory cells. The main common feature of B-cell epitopes is a surface accessibility and antigenicity (40, 41). Therefore, B cell epitopes were analyzed using different prediction tools from Immune Epitope Database IEDB analysis resource (**http://www.iedb.org/bcell**/) (42). Bepipred linear epitope prediction tool (43) was used to predict the linear B-cell epitopes from with a threshold value of 0.064. The conservancy of predicted epitopes were tested using BioEdit program and only conserved epitopes were selected for further analysis. After that, Emini surface accessibility prediction tool (44) and Kolaskar and Tongaonkar antigenicity method (45) were used to predict surface accessibility and antigenicity with threshold values of 1.000 and 1.017 respectively. Epitopes which pass these tests were predicted as B cell epitope.

T cell epitopes prediction:

T-cell epitopes are short, linear peptide sequences that are recognized by T cell receptor through their binding with major histocompatibility complex (MHC) to initiate the T cell response (46-48).

Binding predictions for MHC class I:

IEDB MHC I binding prediction tool (**http://tools.iedb.org/mhci/**) was used to predict the peptide binding to MHC1 molecules. The artificial neural network (ANN) method was used to predict the attachment of cleaved peptides to MHC molecules (42, 49). All epitope lengths were set as 9 mers before prediction. Conserved epitopes that bind to MHC I alleles at score equal or less than 500 half-maximal inhibitory concentrations (IC_{50}) were selected for further analysis (50, 51).

Binding predictions for MHC class II:

To predict binding of peptides to MHC II, IEDB MHC II binding prediction tool (**http://tools.iedb.org/mhcii/**) was used. Human allele references set were used to predict the

binding (52, 53). As MHC II groove can bind peptides having different lengths, epitopes binding prediction is more challenging and less accurate than MHC I binding (54). Similarly to MHC I binding analysis, NN-align method was also used to analyze MHC II binding core epitopes (55). Conserved epitopes that bind to alleles at score equal or less than 1000 half-maximal inhibitory concentration (IC_{50}) were selected for further analysis (50).

Population coverage calculation:

The Population Coverage tool provides a perception about the efficacy of epitopes to regional and global populations (56). Human population coverage for all predicted MHC I and MHC II epitopes was tested against the whole world population, northeast Asia, and southeast Asia - as places where *S. japonicum* is endemic- with the selected MHC I and MHC II interacted alleles using IEDB analysis resource for population coverage calculation (**http://tools.iedb.org/tools/population/iedb_input**) (57).

Assessment of epitope allergenicity:

AllerTop v.2.0 (**http://www.ddg-pharmfac.net/AllerTOP/**) was used to predict the allergenicity of candidate B cell, MHC I and MHC II epitopes. The candidate epitopes were analyzed as either "probable allergen" or "probable non-allergen" meaning that the epitope either cause or does not cause specific IgE production and hypersensitivity responses respectively (58).

Homology modeling:

3D structure of the protein sequence of *Schistosoma japonicum TCTP* was sent to Raptor X (**http/www.raptor.uch icago.edu**) to predict 3D structure of the protein and then treated with UCSF Chimera (version 1.10.2) to visualize the 3D structure. Furthermore, predicted B cell epitopes as well as all predicted T cell epitopes were verified in the structural level (59, 60).

Results

Phylogenetic analysis of the protein sequences:

The relationship between retrieved strains of *TCTP* of *Schistosoma japonicum* is shown in **Figure 2**. The phylogenetic tree divided into two groups. One group contained all strains except strain

CAX71615.1 which found in another group. Therefore, protein strain **CAX71615.1** was outgroup and the rest of strains have a common ancestor. Moreover, strains **AAB42079.1** and **P91800.1** were last strains result from an evolution process.

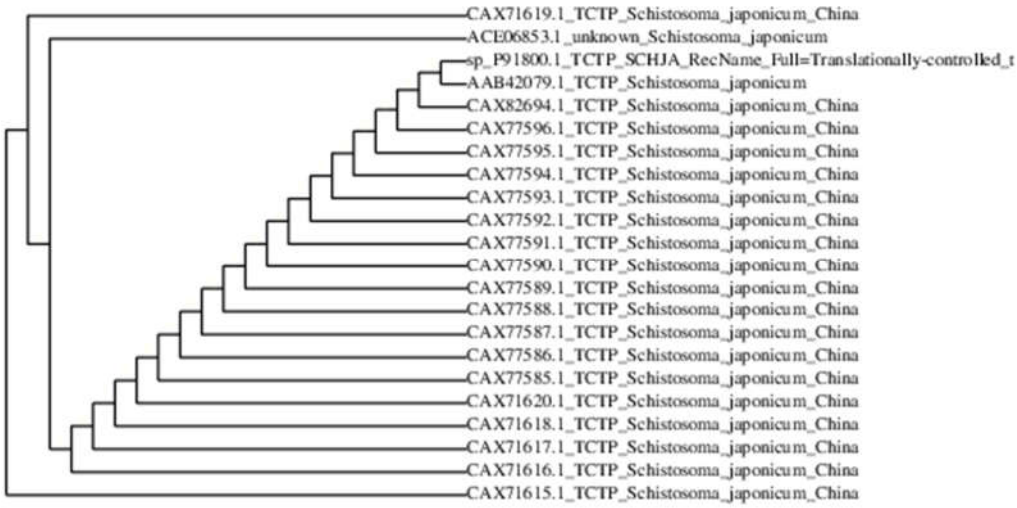

Figure 2. Phylogenetic tree of the retrieved sequences of TCTP of *Schistosoma japonicum* using Phylogeny.fr software.

Protein sequences conservancy analysis:

The use of conserved regions would be efficient in providing broader protection across multiple strains, than epitopes derived from highly variable regions (61, 62). Therefore, retrieved sequences were subjected to conservancy analysis. All the retrieved sequences are highly conserved except two strains **CAX71619.1** and **CAX71615.1** which have mutation at position 127 and 36 respectively as shown in **Figure 3**.

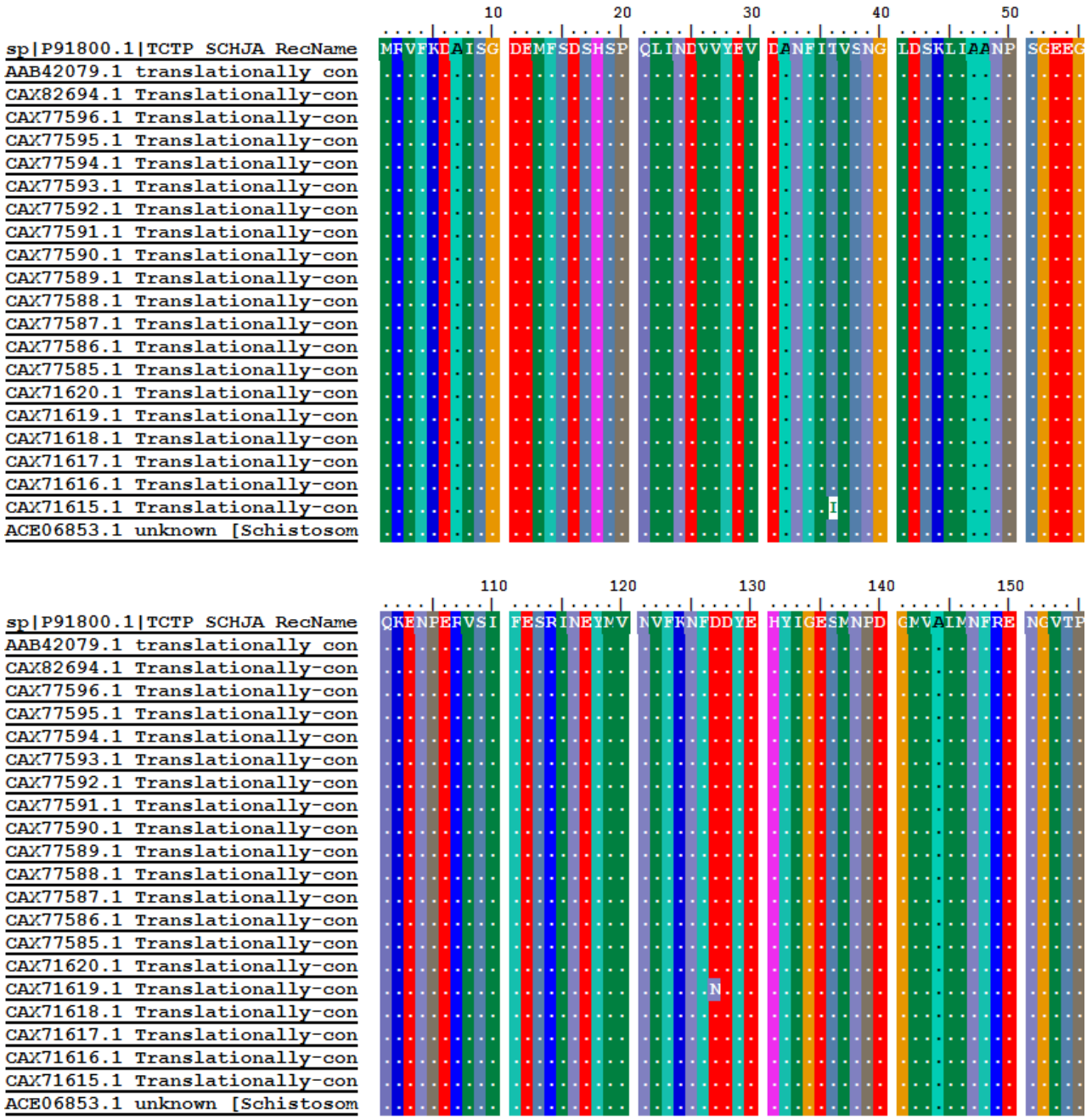

Figure 3. Multiple sequence alignment of whole length amino acid of 22 TCTP strains using BioEdit program (v7.2.5); The highlighted regions represent the mutated region; Dots represent the conservancy between amino acid sequences.

B cell epitopes prediction:

The protein sequence was analyzed using Bepipred linear epitope prediction, Emini surface accessibility and Kolaskar and Tongaonkar antigenicity methods in IEDB to predict the linear

epitope, surface accessibility and to assess the antigenicity respectively. Any value equal or greater than the default threshold 0.064 for linearity, 1.000 for surface accessibility and 1.017 for antigenicity were considered as B cell epitope, **Figure 4-6**. Conserved predicted B cell epitopes are listed in **Table 2**. Three linear peptides **DYEHYI**, **DYEHY,** and **YEHYI,** who have higher prediction scores in surface accessibility and antigenicity, were selected as candidate epitopes, their position in structural level is presented in **Figure 7.**

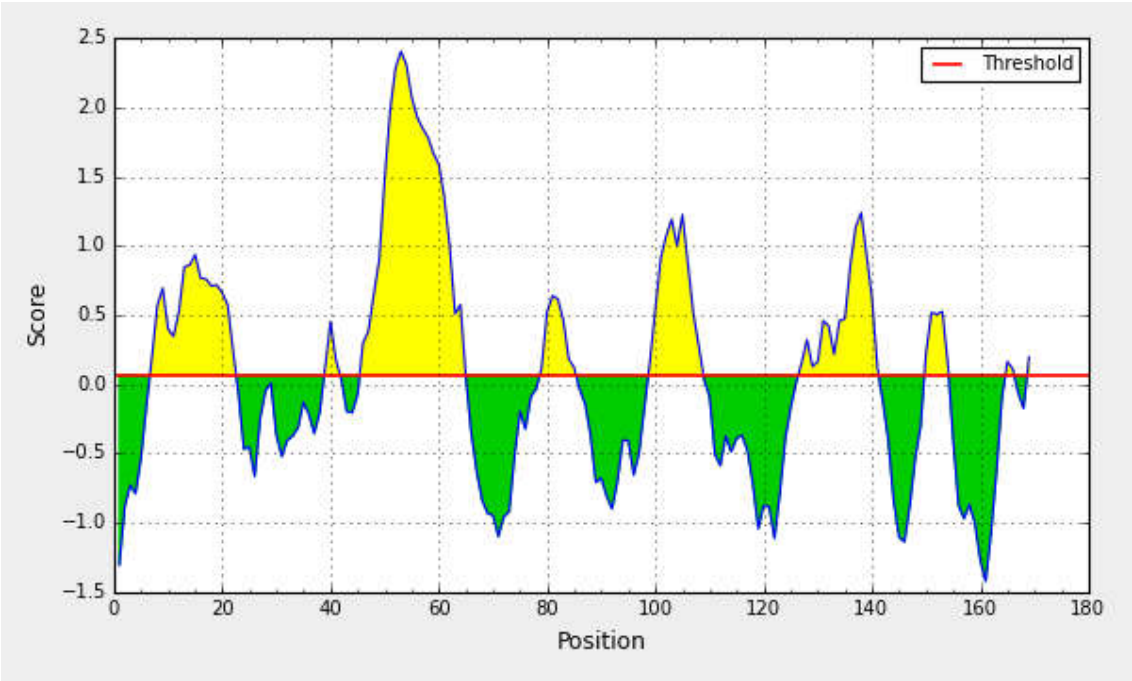

Figure 4. Results of IEDB Bepipred Linear Epitope Prediction tool (**Average**: 0.064 **Minimum**: - 1.425 **Maximum**: 2.407). Yellow areas above threshold (red line) are proposed to be a part of B cell epitopes and the green areas are not.

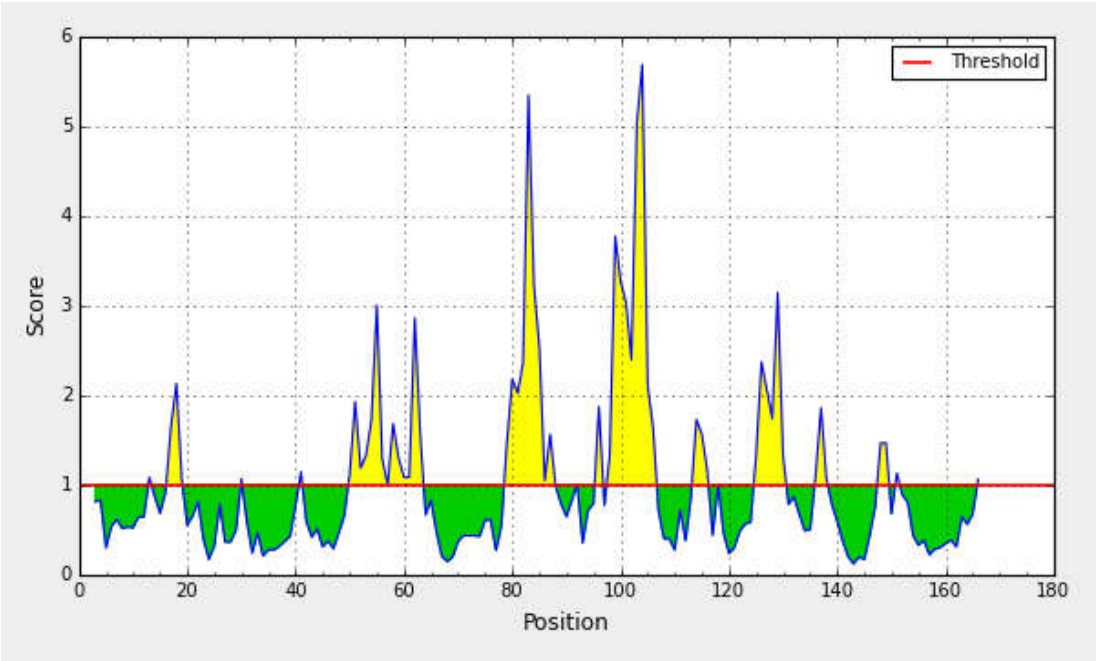

Figure 5. Results of IEDB Emini Surface Accessibility Prediction tool (**Average**: 0.064 **Minimum**: - 1.425 **Maximum**: 2.407). Yellow areas above threshold (red line) are proposed to be a part of B cell epitopes and the green areas are not.

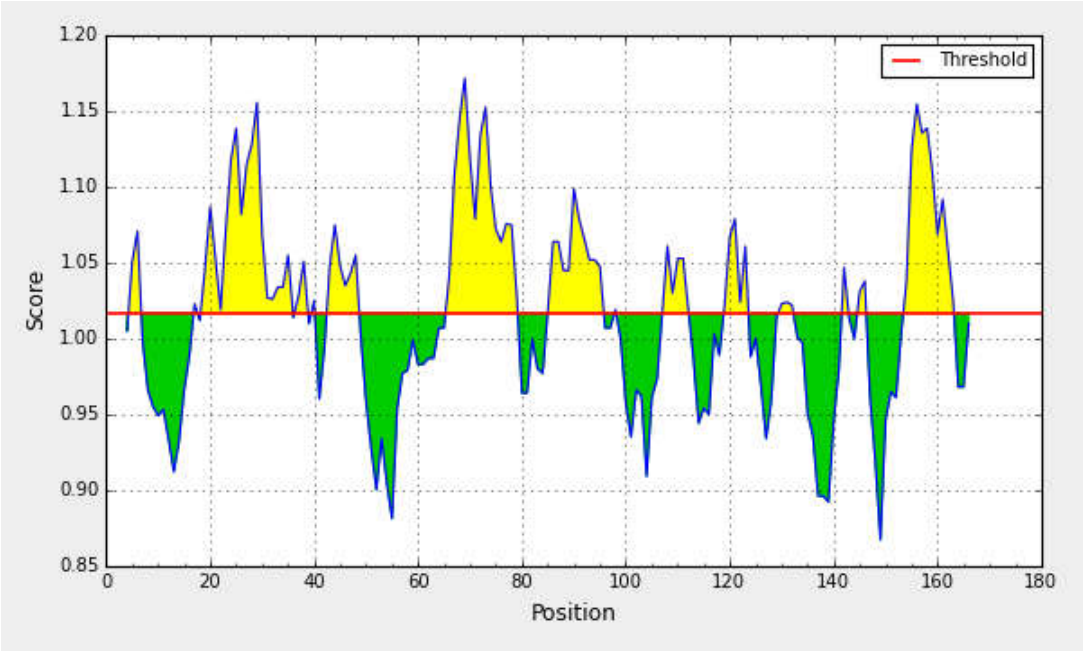

Figure 6. Results of IEDB Kolaskar &Tongaonkar Antigenicity prediction tool (**Average**: 1.017 **Minimum**: 0.867 **Maximum**: 1.172). Yellow areas above threshold (red line) are proposed to be a part of B cell epitopes and the green areas are not.

Table 2. list of B cell epitopes predicted using different scales of *Schistosoma japonicum*.

Start	End	Epitope	Length	Emini surface threshold (1.00)	Antigenicity threshold (1.017)
7	22	AISGDEMFSDSHSPQL	16	0.409	1.005
39	41	NGL	3	0.574	0.967
46	64	IAANPSGEEGQEEVSDSTE	19	1.4	0.965
79	85	SFDKKSY	7	2.441	1
99	108	RLQKENPERV	10	4.029	0.987
128	141	***DYEHYIGESMNPDG**	14	1.533	0.96
128	140	DYEHYIGESMNPD	13	1.994	0.967
128	139	DYEHYIGESMNP	12	1.544	0.975
128	138	DYEHYIGESMN	11	1.274	0.967
128	137	DYEHYIGESM	10	1.023	0.986
128	136	DYEHYIGES	9	1.351	1.004
128	135	DYEHYIGE	8	1.331	1.003
129	136	YEHYIGES	8	1.068	1.021
128	134	DYEHYIG	7	1.005	1.024
129	135	YEHYIGE	7	1.042	1.022
128	133	***DYEHYI**	6	1.321	1.049
128	132	***DYEHY**	5	2.511	1.029
129	133	***YEHYI**	5	1.054	1.086
129	132	YEHY	4	1.97	1.07
129	131	YEH	3	1.616	1.039
150	154	ENGVT	5	0.767	0.959
165	166	IE	2	0.692	1.002

***Peptide** from (128 to 141) gives a good score in Kolaskar & Tongaonkar antigenicity if it is shortened to 6 amino acids (128 to 133) or to 5 amino acid (129 to 133) & 5 amino acid (129 to 133).

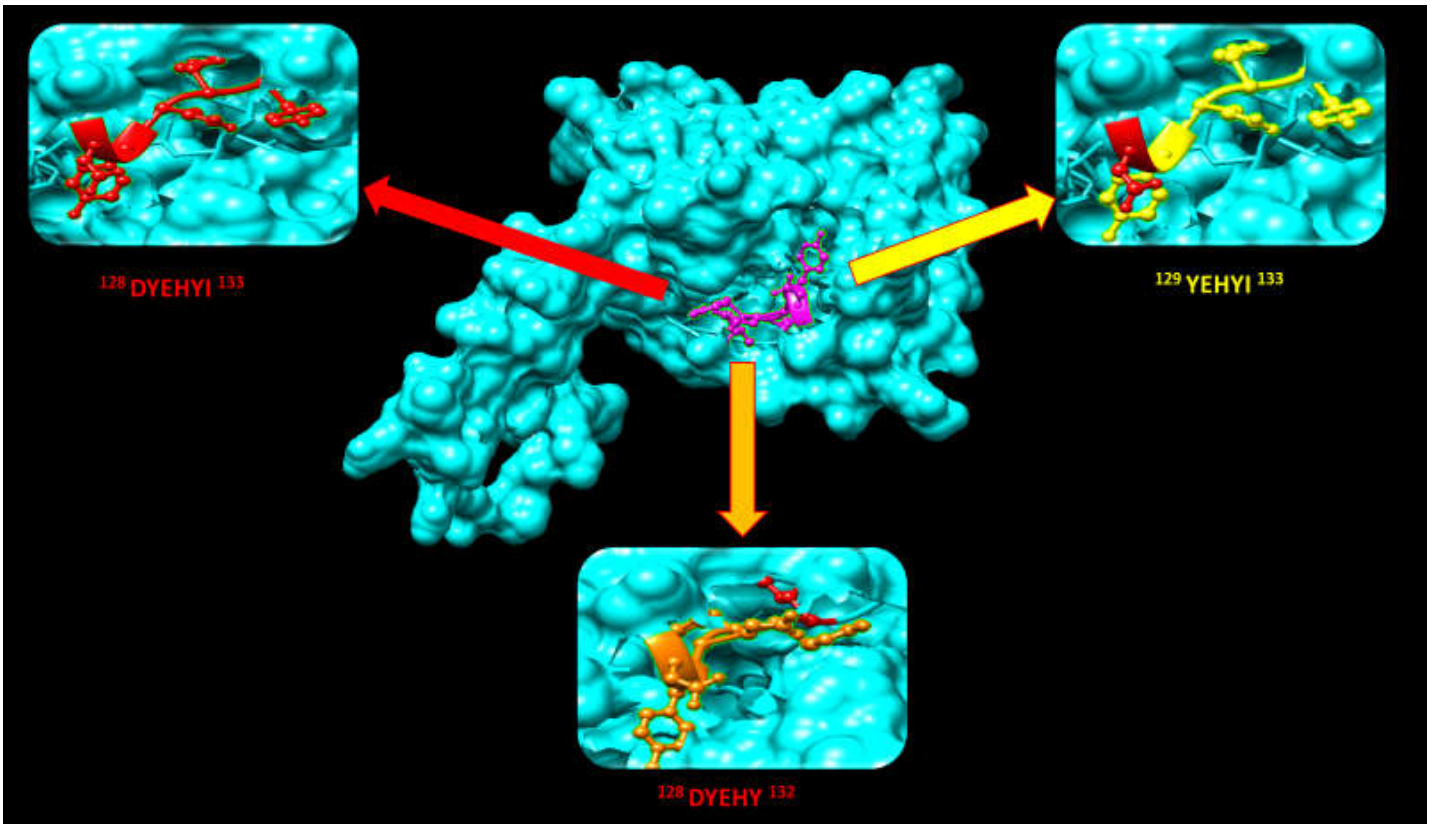

Figure 7. Structural visualization of proposed B cell epitopes of *S. japonicum TCTP* and their overlapping position in the protein from 128 to 133 using UCSF Chimera (version 1.10.2).

Binding predictions for MHC class I:

Schistosoma japonicum TCTP was analyzed using IEDB MHC I binding prediction tool. Based on ANN-method, 54 conserved peptides were predicted to interact with different types of MHC I alleles with $IC_{50} \leq 500$nM. Four epitopes, **YMVNVFKNF** (118-126), **VVYEVDANF** (26-34), **YEHYIGESM** (129-137), and **FRENGVTPY** (148-156) were found to interact with large number of MHC I alleles with high and intermediate affinity, **Table 3.** Their position in structural level is presented in **Figure 8.**

Table 3. List of epitopes that bind to MHC Class I alleles with high and intermediate affinity

Peptide	Start	End	Length	Allele	ANN_ic50	Percentile
YMVNVFKNF	118	126	9	HLA-A*02:06	186.59	0.2
				HLA-A*23:01	27.97	0.2
				HLA-A*29:02	229.86	0.2
				HLA-B*15:01	17.3	0.1
				HLA-B*15:02	83.03	0.1
				HLA-C*12:03	139.04	0.2
				HLA-C*14:02	56.72	0.2
VVYEVDANF	26	34	9	HLA-A*02:06	239.87	0.2
				HLA-A*23:01	167.66	0.2
				HLA-B*15:01	167.59	0.2
				HLA-B*15:02	478.94	0.1
				HLA-B*35:01	332.42	0.3
				HLA-B*58:01	443.26	0.2
				HLA-C*12:03	379.95	0.2
				HLA-C*14:02	365.36	0.2
YEHYIGESM	129	137	9	HLA-B*15:01	456.89	0.2
				HLA-B*18:01	8.38	0.1
				HLA-B*40:01	9.59	0.1
				HLA-B*40:02	40.95	0.1
				HLA-C*03:03	78.58	0.2
				HLA-C*12:03	192.74	0.2
				HLA-C*14:02	49.08	0.2
FRENGVTPY	148	156	9	HLA-B*15:02	131.87	0.1
				HLA-B*35:01	414.47	0.3
				HLA-C*06:02	499.61	0.1
				HLA-C*07:01	403.71	9.7
				HLA-C*07:02	68.06	0.1
				HLA-C*12:03	43.3	0.2

HLA-C*14:02	200.98	0.2

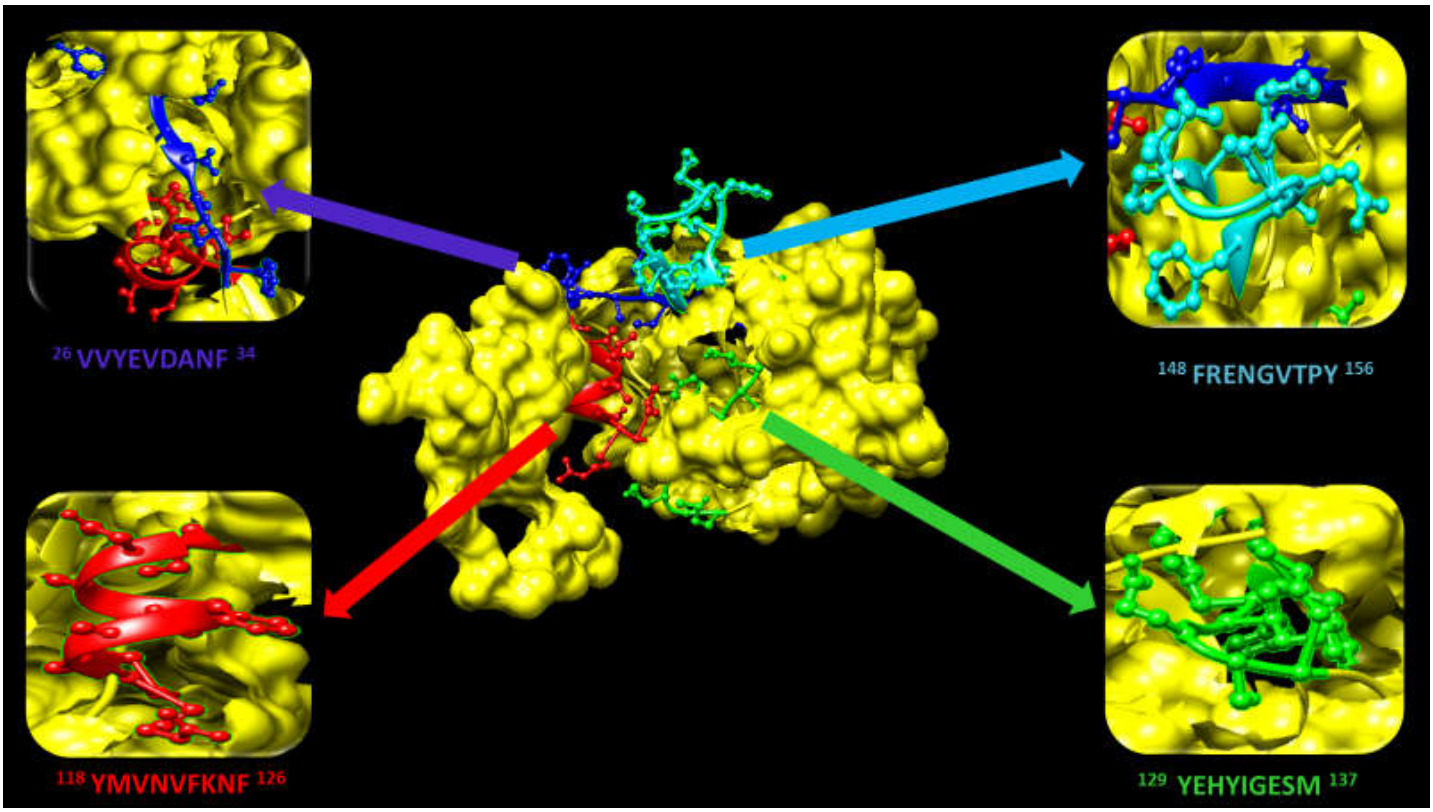

Figure 8. Position of proposed T cell epitopes of *S. japonicum TCTP* that interact with MHC I in structural level using UCSF Chimera (version 1.10.2).

Binding predictions for MHC class II:

96 conserved peptides were predicted to interact with different alleles of MHC II after analyzing the protein sequence using IEDB MHC II binding prediction tool based on NN-align method with an $IC_{50} \leq 1000$nM. The core peptides, **IDLVHASRL** had high affinity to interact with twelve alleles, **YLKAIKERL** interacted with sixteen alleles, however **YLKGYLKAI** was found to interact with eleven alleles, **Table 4** and **Figure 9**. Several overlapping between MHC class I and MHC Class II epitopes were found. These overlapping between MHC I and MHC II epitopes are illustrated in **Table 5**.

Table4. List of epitopes that bind to large number of MHC Class II alleles.

Core Peptide	Start	End	Allele	Peptide Sequence	IC50	Rank
IDLVHASRL	67	75	HLA-DPA1*02:01/DPB1*01:01	ERVIDLVHASRLVST	346.3	25.09
				RVIDLVHASRLVSTS	352.6	25.36
				TERVIDLVHASRLVS	353.6	25.4
				VIDLVHASRLVSTSF	364.1	25.83
				STERVIDLVHASRLV	437.6	28.64
				IDLVHASRLVSTSFD	463.2	29.52
				DSTERVIDLVHASRL	540.7	32
			HLA-DPA1*03:01/DPB1*04:02	VIDLVHASRLVSTSF	305.3	18.54
				ERVIDLVHASRLVST	310.7	18.71
				RVIDLVHASRLVSTS	319	18.97
				TERVIDLVHASRLVS	390.8	20.98
				IDLVHASRLVSTSFD	421.5	21.77
				STERVIDLVHASRLV	437.2	22.17
				DSTERVIDLVHASRL	560.9	24.89
			HLA-DRB1*01:01	ERVIDLVHASRLVST	14.7	8.42
				TERVIDLVHASRLVS	18.3	10.44
				RVIDLVHASRLVSTS	18.4	10.49
				STERVIDLVHASRLV	23.3	12.82
				DSTERVIDLVHASRL	32.3	16.25
				VIDLVHASRLVSTSF	33	16.49
				IDLVHASRLVSTSFD	47.3	20.58
			HLA-DRB1*03:01	ERVIDLVHASRLVST	68.1	3.71
				TERVIDLVHASRLVS	70.3	3.8
				STERVIDLVHASRLV	87.6	4.51
				DSTERVIDLVHASRL	197.2	7.98
			HLA-DRB1*04:05	STERVIDLVHASRLV	448.7	27.59
				TERVIDLVHASRLVS	467	28.22
				ERVIDLVHASRLVST	470.7	28.33

	DSTERVIDLVHASRL	561.1	31.12
	RVIDLVHASRLVSTS	639.7	33.31
HLA-DRB1*07:01	STERVIDLVHASRLV	3	0.1
	TERVIDLVHASRLVS	3.1	0.11
	ERVIDLVHASRLVST	3.3	0.15
	DSTERVIDLVHASRL	3.6	0.2
	RVIDLVHASRLVSTS	3.7	0.21
	VIDLVHASRLVSTSF	4.9	0.48
	IDLVHASRLVSTSFD	6.5	0.83
HLA-DRB1*09:01	ERVIDLVHASRLVST	82.7	5.72
	TERVIDLVHASRLVS	100.4	6.96
	RVIDLVHASRLVSTS	119.1	8.18
	VIDLVHASRLVSTSF	133.1	9.06
	STERVIDLVHASRLV	145.9	9.86
	DSTERVIDLVHASRL	211.4	13.54
HLA-DRB1*11:01	DSTERVIDLVHASRL	297.7	24.67
HLA-DRB1*13:02	TERVIDLVHASRLVS	27.4	1.99
	STERVIDLVHASRLV	28	2.03
	ERVIDLVHASRLVST	28.7	2.08
	DSTERVIDLVHASRL	37.6	2.69
	RVIDLVHASRLVSTS	48.4	3.38
	VIDLVHASRLVSTSF	80.8	5.2
	IDLVHASRLVSTSFD	159.9	8.47
HLA-DRB1*15:01	ERVIDLVHASRLVST	41.1	4.05
	TERVIDLVHASRLVS	42.3	4.19
	STERVIDLVHASRLV	50.7	5.17
	RVIDLVHASRLVSTS	51.2	5.22
	DSTERVIDLVHASRL	78.9	8.04
	VIDLVHASRLVSTSF	84.5	8.56
	IDLVHASRLVSTSFD	158.3	14.34
HLA-DRB4*01:01	VIDLVHASRLVSTSF	262.9	17.67

				TERVIDLVHASRLVS	289.5	18.98
				RVIDLVHASRLVSTS	300.7	19.5
				ERVIDLVHASRLVST	306.2	19.75
				IDLVHASRLVSTSFD	329.2	20.78
				STERVIDLVHASRLV	349	21.64
				DSTERVIDLVHASRL	372.5	22.62
			HLA-DRB5*01:01	TERVIDLVHASRLVS	59.4	11.18
				STERVIDLVHASRLV	69.8	12.38
				ERVIDLVHASRLVST	74.9	12.94
				DSTERVIDLVHASRL	102.5	15.51
				RVIDLVHASRLVSTS	129.5	17.62
				VIDLVHASRLVSTSF	339	28.05
				IDLVHASRLVSTSFD	977.1	42.87
YLKAIKERL	92	100	HLA-DPA1*01:03/DPB1*02:01	LKGYLKAIKERLQKE	881.1	28.64
			HLA-DPA1*02:01/DPB1*01:01	RAYLKGYLKAIKERL	138.6	13.57
				LKGYLKAIKERLQKE	138.6	13.57
				KGYLKAIKERLQKEN	152.2	14.55
				AYLKGYLKAIKERLQ	163.1	15.33
				YLKGYLKAIKERLQK	187.4	16.96
				GYLKAIKERLQKENP	223.5	19.12
				YLKAIKERLQKENPE	244.6	20.28
			HLA-DPA1*02:01/DPB1*05:01	LKGYLKAIKERLQKE	458.1	9.62
				KGYLKAIKERLQKEN	844.9	15.72
			HLA-DPA1*03:01/DPB1*04:02	LKGYLKAIKERLQKE	798.1	29.02
				KGYLKAIKERLQKEN	945.6	31.14
			HLA-DRB1*01:01	LKGYLKAIKERLQKE	34.6	17.01
				YLKGYLKAIKERLQK	39.8	18.58
			HLA-DRB1*03:01	LKGYLKAIKERLQKE	112.7	5.47
				YLKGYLKAIKERLQK	159.6	6.95
				KGYLKAIKERLQKEN	265	9.56
				AYLKGYLKAIKERLQ	280.6	9.91

	RAYLKGYLKAIKERL	477.7	13.74
	GYLKAIKERLQKENP	667.1	16.52
HLA-DRB1*04:01	LKGYLKAIKERLQKE	145.9	11.51
	YLKGYLKAIKERLQK	160.2	12.48
	AYLKGYLKAIKERLQ	198.5	14.9
	YLKAIKERLQKENPE	467.8	27.64
HLA-DRB1*04:04	KGYLKAIKERLQKEN	477.1	33.9
	LKGYLKAIKERLQKE	524.6	35.54
HLA-DRB1*04:05	AYLKGYLKAIKERLQ	84.7	8.31
	YLKGYLKAIKERLQK	96.3	9.31
	LKGYLKAIKERLQKE	110.4	10.47
	KGYLKAIKERLQKEN	228.8	18.18
	GYLKAIKERLQKENP	433.8	27.07
	YLKAIKERLQKENPE	931.4	40.05
HLA-DRB1*07:01	RAYLKGYLKAIKERL	66.8	10.76
	AYLKGYLKAIKERLQ	72.3	11.4
	YLKGYLKAIKERLQK	97.6	13.96
	LKGYLKAIKERLQKE	153.7	18.48
	KGYLKAIKERLQKEN	260.3	24.48
	GYLKAIKERLQKENP	498	33.23
	YLKAIKERLQKENPE	659.4	37.47
HLA-DRB1*08:02	LKGYLKAIKERLQKE	270.6	6.27
HLA-DRB1*09:01	LKGYLKAIKERLQKE	69.5	4.72
	RAYLKGYLKAIKERL	79.9	5.5
	AYLKGYLKAIKERLQ	93.2	6.46
	KGYLKAIKERLQKEN	93.5	6.48
	YLKGYLKAIKERLQK	96.2	6.65
	GYLKAIKERLQKENP	171.6	11.38
	YLKAIKERLQKENPE	408.1	22.27
HLA-DRB1*11:01	LKGYLKAIKERLQKE	9.1	1.11
	YLKGYLKAIKERLQK	11.5	1.68

				KGYLKAIKERLQKEN	11.5	1.68
				AYLKGYLKAIKERLQ	17.2	2.96
				GYLKAIKERLQKENP	17.9	3.13
				RAYLKGYLKAIKERL	23	4.2
				YLKAIKERLQKENPE	32	5.86
			HLA-DRB1*15:01	YLKGYLKAIKERLQK	59.7	6.14
				LKGYLKAIKERLQKE	88.3	8.9
				KGYLKAIKERLQKEN	411.2	26.23
				GYLKAIKERLQKENP	737.6	35.12
			HLA-DRB4*01:01	RAYLKGYLKAIKERL	545.6	28.95
			HLA-DRB5*01:01	YLKGYLKAIKERLQK	5.3	0.93
				LKGYLKAIKERLQKE	5.4	0.97
				AYLKGYLKAIKERLQ	5.7	1.07
				KGYLKAIKERLQKEN	8.3	1.86
				GYLKAIKERLQKENP	14	3.46
				YLKAIKERLQKENPE	24.5	5.84
YLKGYLKAI	88	96	HLA-DPA1*01/DPB1*04:01	KSYRAYLKGYLKAIK	273.2	10.37
				SYRAYLKGYLKAIKE	284.9	10.65
				YRAYLKGYLKAIKER	285.7	10.67
				KKSYRAYLKGYLKAI	300.3	11
				RAYLKGYLKAIKERL	417.4	13.4
				AYLKGYLKAIKERLQ	772.7	18.97
			HLA-DPA1*01:03/DPB1*02:01	KKSYRAYLKGYLKAI	119.6	9.36
				KSYRAYLKGYLKAIK	120	9.38
				SYRAYLKGYLKAIKE	137.9	10.26
				YRAYLKGYLKAIKER	164.8	11.45
				RAYLKGYLKAIKERL	270.4	15.35
				AYLKGYLKAIKERLQ	514.5	21.81
				YLKGYLKAIKERLQK	868.2	28.43
			HLA-DPA1*02:01/DPB1*01:01	SYRAYLKGYLKAIKE	180	16.48
				YRAYLKGYLKAIKER	181	16.55

	KSYRAYLKGYLKAIK	185.1	16.81
	KKSYRAYLKGYLKAI	194.4	17.38
HLA-DPA1*02:01/DPB1*05:01	YRAYLKGYLKAIKER	106.3	2.16
	RAYLKGYLKAIKERL	150.4	3.25
	AYLKGYLKAIKERLQ	218.6	4.83
	YLKGYLKAIKERLQK	297.3	6.53
HLA-DPA1*03:01/DPB1*04:02	YRAYLKGYLKAIKER	315.2	18.85
	SYRAYLKGYLKAIKE	339.5	19.58
	RAYLKGYLKAIKERL	400.1	21.23
	KSYRAYLKGYLKAIK	473.6	23.02
	AYLKGYLKAIKERLQ	497.8	23.57
	KKSYRAYLKGYLKAI	686.9	27.22
	YLKGYLKAIKERLQK	828	29.48
HLA-DQA1*05:01/DQB1*03:01	SYRAYLKGYLKAIKE	183.6	21.52
	YRAYLKGYLKAIKER	183.7	21.53
	KSYRAYLKGYLKAIK	197.4	22.41
	RAYLKGYLKAIKERL	217.8	23.67
	AYLKGYLKAIKERLQ	240.6	24.95
	KKSYRAYLKGYLKAI	256.5	25.8
	YLKGYLKAIKERLQK	306	28.31
HLA-DRB1*01:01	YRAYLKGYLKAIKER	11.4	6.24
	SYRAYLKGYLKAIKE	18.3	10.44
	RAYLKGYLKAIKERL	20.8	11.69
	KSYRAYLKGYLKAIK	20.9	11.74
	KKSYRAYLKGYLKAI	34.8	17.08
	AYLKGYLKAIKERLQ	42.4	19.3
HLA-DRB1*04:01	YRAYLKGYLKAIKER	510.5	29.2
	SYRAYLKGYLKAIKE	633.5	33.28
	KSYRAYLKGYLKAIK	699.8	35.26
	KKSYRAYLKGYLKAI	843.5	39.19
HLA-DRB1*04:04	RAYLKGYLKAIKERL	339.7	28.31

	YRAYLKGYLKAIKER	479.9	33.98
	KSYRAYLKGYLKAIK	989.9	47
HLA-DRB1*07:01	KKSYRAYLKGYLKAI	270.3	24.93
	KSYRAYLKGYLKAIK	441.9	31.5
	SYRAYLKGYLKAIKE	495.2	33.15
HLA-DRB1*09:01	YRAYLKGYLKAIKER	133.9	9.11
	SYRAYLKGYLKAIKE	155.9	10.44
	KSYRAYLKGYLKAIK	174.9	11.57

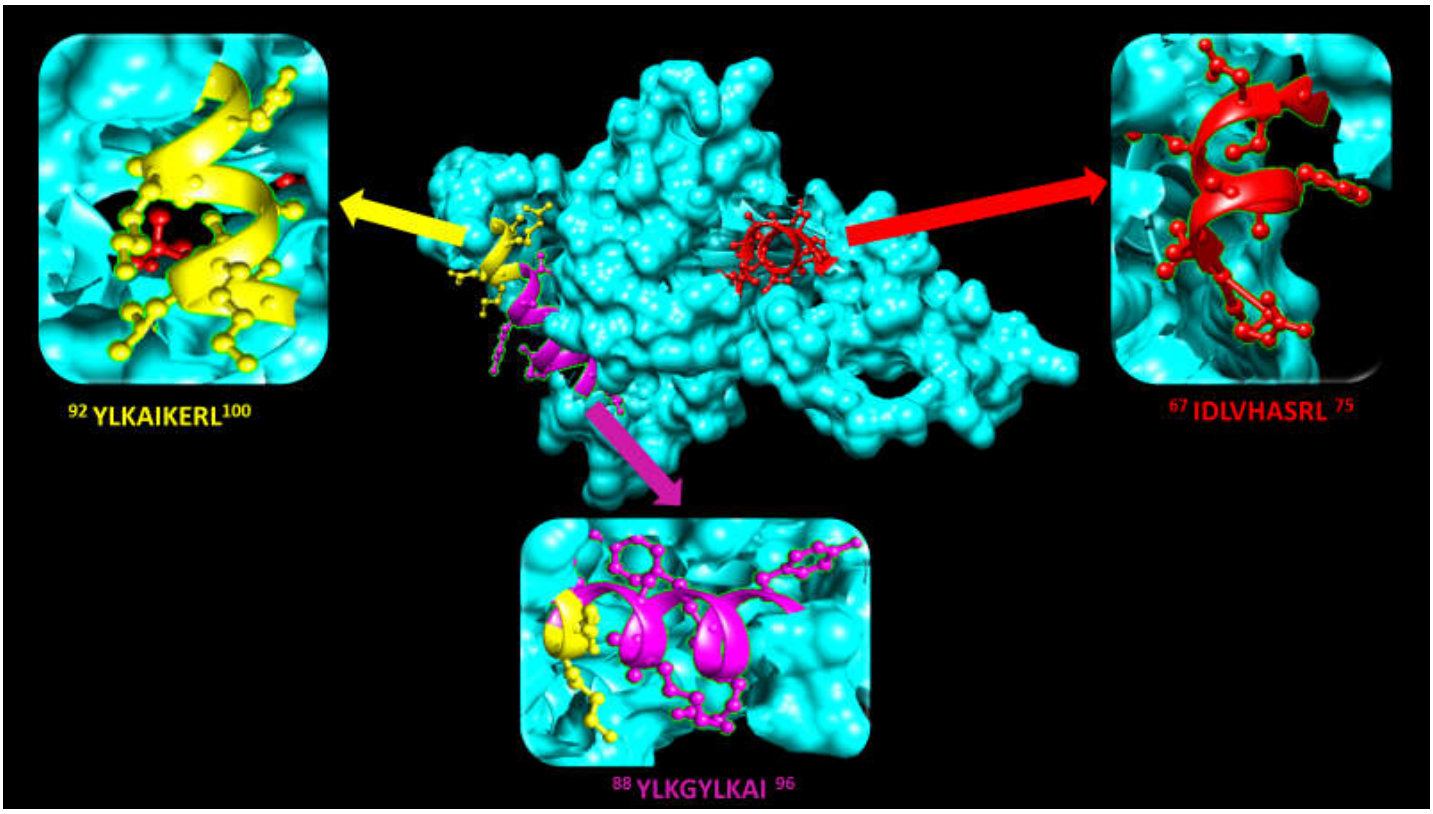

Figure 9. Position of proposed T cell epitopes of *S. japonicum TCTP* that interact with MHC II in structural level using UCSF Chimera (version 1.10.2).

Table 5. Overlapping between MHC class I and II epitopes.

Epitope (MHC II)	Peptide Sequence	Start	End	Epitope (MHC I)
AYLKGYLKA	DK**KSYRAYLKG**YLKA	81	95	**KSYRAYLKG**

DKKSYRAYL	TSFDK**KSYRAYLKG**Y	78	92	
FDKKSYRAY	STSFDK**KSYRAYLKG**	77	91	
KKSYRAYLK	FDK**KSYRAYLKG**YLK	80	94	
LKGYLKAIK	**KSYRAYLKG**YLKAIK	83	97	
RAYLKGYLK	DK**KSYRAYLKG**YLKA	81	95	
YLKGYLKAI	**KSYRAYLKG**YLKAIK	83	97	
YRAYLKGYL	**KSYRAYLKG**YLKAIK	83	97	
LQKENPERV	KE**RLQKENPER**VSIF	97	111	**RLQKENPER**
LKAIKERLQ	LKAIKE**RLQKENPER**	93	107	
KAIKERLQK	KAIKE**RLQKENPER**V	94	108	
ENPERVSIF	**RLQKENPER**VSIFES	99	113	
AIKERLQKE	K**GYLKAIKER**LQKEN	90	104	**GYLKAIKER**
KGYLKAIKE	AYLK**GYLKAIKER**LQ	87	101	
LKAIKERLQ	K**GYLKAIKER**LQKEN	90	104	
LKGYLKAIK	AYLK**GYLKAIKER**LQ	87	101	
RAYLKGYLK	YRAYLK**GYLKAIKER**	85	99	
YLKAIKERL	AYLK**GYLKAIKER**LQ	87	101	
YLKGYLKAI	YRAYLK**GYLKAIKER**	85	99	
YRAYLKGYL	YRAYLK**GYLKAIKER**	85	99	
EVSDSTERV	Q**EEVSDSTER**VIDLV	56	70	**EEVSDSTER**
VSDSTERVI	EEGQ**EEVSDSTER**VI	53	67	
DGMVALMNF	**ESMNPDGMV**ALMNFR	135	149	**ESMNPDGMV**
GMVALMNFR	**ESMNPDGMV**ALMNFR	135	149	
MNPDGMVAL	**ESMNPDGMV**ALMNFR	135	149	
NPDGMVALM	IG**ESMNPDGMV**ALMN	133	147	
PDGMVALMN	**ESMNPDGMV**ALMNFR	135	149	
YIGESMNPD	YEHYIG**ESMNPDGMV**	129	143	
DLVHASRLV	**STERVIDLV**HASRLV	62	76	**STERVIDLV**
DSTERVIDL	SD**STERVIDLV**HASR	60	74	
ERVIDLVHA	SD**STERVIDLV**HASR	60	74	
EVSDSTERV	QEEVSD**STERVIDLV**	56	70	

IDLVHASRL	**STERVIDLV**HASRLV	62	76	
RVIDLVHAS	**STERVIDLV**HASRLV	62	76	
VIDLVHASR	**STERVIDLV**HASRLV	62	76	
FSDSHSPQL	EMFSDS**HSPQLINDV**	12	26	**HSPQLINDV**
INDVVYEVD	**HSPQLINDV**VYEVDA	18	32	
LINDVVYEV	**HSPQLINDV**VYEVDA	18	32	
MFSDSHSPQ	EMFSDS**HSPQLINDV**	12	26	
QLINDVVYE	**HSPQLINDV**VYEVDA	18	32	
SPQLINDVV	**HSPQLINDV**VYEVDA	18	32	
DGMVALMNF	E**SMNPDGMVA**LMNFR	135	149	**SMNPDGMVA**
GMVALMNFR	E**SMNPDGMVA**LMNFR	135	149	
MNPDGMVAL	**SMNPDGMVA**LMNFRE	136	150	
MVALMNFRE	**SMNPDGMVA**LMNFRE	136	150	
NPDGMVALM	IGE**SMNPDGMVA**LMN	133	147	
PDGMVALMN	**SMNPDGMVA**LMNFRE	136	150	
YIGESMNPD	EHYIGESMNPDGMVA	130	144	
DGMVALMNF	**GESMNPDGM**VALMNF	134	148	**GESMNPDGM**
MNPDGMVAL	**GESMNPDGM**VALMNF	134	148	
NPDGMVALM	I**GESMNPDGM**VALMN	133	147	
PDGMVALMN	**GESMNPDGM**VALMNF	134	148	
YIGESMNPD	DYEHYI**GESMNPDGM**	128	142	
ENGVTPYFV	F**RENGVTPYF**VFLKD	148	162	**RENGVTPYF**
FRENGVTPY	LMNF**RENGVTPYF**VF	145	159	
GVTPYFVFL	NF**RENGVTPYF**VFLK	147	161	
LMNFRENGV	VALMNF**RENGVTPYF**	143	157	
NGVTPYFVF	LMNF**RENGVTPYF**VF	145	159	
TPYFVFLKD	**RENGVTPYF**VFLKDG	149	163	
VALMNFREN	VALMNF**RENGVTPYF**	143	157	
VTPYFVFLK	**RENGVTPYF**VFLKDG	149	163	
ENPERVSIF	LQ**KENPERVSI**FESR	100	114	**KENPERVSI**
ERVSIFESR	Q**KENPERVSI**FESRI	101	115	

LQKENPERV	KERLQ**KENPERVSI**F	97	111	
PERVSIFES	**KENPERVSI**FESRIN	102	116	
RVSIFESRI	**KENPERVSI**FESRIN	102	116	
VSIFESRIN	**KENPERVSI**FESRIN	102	116	
GEEGQEEVS	I**AANPSGEEG**QEEVS	46	60	**AANPSGEEG**
KLIAANPSG	LDSKLI**AANPSGEEG**	41	55	
LDSKLIAAN	LDSKLI**AANPSGEEG**	41	55	
LIAANPSGE	LDSKLI**AANPSGEEG**	41	55	
PSGEEGQEE	KLI**AANPSGEEG**QEE	44	58	
SKLIAANPS	LDSKLI**AANPSGEEG**	41	55	

Population coverage analysis:

All predicted T cell epitopes that are interacted with MHC I and MHC II were used for population coverage analysis against the whole world population. The results of population coverage of all epitopes are listed in **Table 6** and **7.** Proposed epitopes which interacted with large number of HLA class I and class II alleles were analyzed against the whole world population, northeast Asia, and southeast Asia. Results of that analysis are presented in **Table 8** and **9**.

Table 6. Population coverage of all epitopes in MHC class I in the world.

Epitope	Coverage class I	Total HLA hits	Epitope	Coverage class I	Total HLA hits
LINDVVYEV	45.07%	4	**GYLKAIKER**	5.36%	1
RINEYMVNV	46.61%	4	**VIDLVHASR**	5.36%	1
GLDSKLIAA	40.60%	2	**NEYMVNVFK**	12.72%	2
YLKGYLKAI	53.88%	5	**ERVSIFESR**	5.83%	1
GVTPYFVFL	45.42%	3	**EEVSDSTER**	5.83%	1
LMNFRENGV	39.08%	1	**EVSDSTERV**	2.50%	1
RVIDLVHAS	1.95%	1	**ESMNPDGMV**	6.81%	2
YMVNVFKNF	31.13%	7	**STERVIDLV**	2.50%	1
VVYEVDANF	37.10%	8	**HSPQLINDV**	2.50%	1
FVFLKDGLI	4.43%	2	**SPQLINDVV**	12.78%	1

FSDSHSPQL	27.03%	6	**YLKAIKERL**	10.55%	1
FLKDGLIEE	1.95%	1	**ASRLVSTSF**	8.44%	1
RAYLKGYLK	43.03%	5	**SMNPDGMVA**	8.44%	1
RLVSTSFDK	38.48%	4	**YEHYIGESM**	41.07%	7
VTPYFVFLK	43.03%	5	**RENGVTPYF**	37.33%	6
LVSTSFDKK	35.75%	3	**FDKKSYRAY**	12.52%	2
TSFDKKSYR	25.64%	3	**FRENGVTPY**	64.07%	7
KAIKERLQK	19.09%	2	**FESRINEYM**	18.02%	3
GMVALMNFR	25.64%	3	**YRAYLKGYL**	36.50%	3
VYEVDANFI	21.38%	1	**NPDGMVALM**	13.43%	3
IFESRINEY	3.89%	1	**DAISGDEMF**	8.42%	1
SYRAYLKGY	14.91%	5	**GESMNPDGM**	7.81%	1
KSYRAYLKG	10.86%	3	**KENPERVSI**	18.29%	3
KLIAANPSG	3.89%	1	**IAANPSGEE**	8.12%	1
LVHASRLVS	3.89%	1	**AANPSGEEG**	8.12%	1
STSFDKKSY	2.43%	1	**VSDSTERVI**	7.85%	1
RLQKENPER	5.36%	1	**MNPDGMVAL**	13.19%	2
Epitope set	**99.27%**				

Table 7. Population coverage of all epitopes in MHC class II in the world.

Epitope	Coverage class II	Total HLA hits	Epitope	Coverage class II	Total HLA hits
AIKERLQKE	0.00%	1	**LVSTSFDKK**	10.54%	5
AISGDEMFS	0.00%	1	**MFSDSHSPQ**	11.21%	2
ASRLVSTSF	24.24%	4	**MNPDGMVAL**	0.00%	2
AYLKGYLKA	0.00%	1	**MRVFKDAIS**	17.84%	3
DAISGDEMF	0.00%	3	**MVALMNFRE**	52.03%	8
DGMVALMNF	0.00%	3	**NEYMVNVFK**	11.53%	3
DKKSYRAYL	24.18%	2	**NGVTPYFVF**	0.00%	2
DLVHASRLV	11.53%	2	**NPDGMVALM**	0.00%	1
DSTERVIDL	0.00%	1	**PDGMVALMN**	11.53%	3

DVVYEVDAN	0.00%	1	**PERVSIFES**	0.00%	1
ENGVTPYFV	17.55%	3	**PQLINDVVY**	4.77%	1
ENPERVSIF	0.00%	1	**PSGEEGQEE**	0.00%	1
ERVIDLVHA	4.77%	1	**PYFVFLKDG**	0.00%	1
ERVSIFESR	0.00%	1	**QLINDVVYE**	0.00%	1
ESRINEYMV	0.00%	1	**RAYLKGYLK**	28.63%	3
EVSDSTERV	6.69%	2	**RINEYMVNV**	7.71%	4
EYMVNVFKN	0.00%	4	**RLVSTSFDK**	18.23%	6
FDKKSYRAY	11.53%	2	**RVIDLVHAS**	15.70%	3
FESRINEYM	38.20%	4	**RVSIFESRI**	32.51%	7
FKDAISGDE	9.32%	4	**SDSHSPQLI**	11.53%	2
FLKDGLIEE	23.63%	7	**SFDKKSYRA**	11.53%	2
FRENGVTPY	63.66%	10	**SKLIAANPS**	16.02%	4
FSDSHSPQL	24.01%	4	**SNGLDSKLI**	11.53%	2
FVFLKDGLI	49.06%	10	**SPQLINDVV**	0.00%	1
GEEGQEEVS	0.00%	1	**SRINEYMVN**	0.00%	1
GLDSKLIAA	0.00%	2	**SRLVSTSFD**	14.37%	3
GMVALMNFR	18.41%	3	**STSFDKKSY**	10.54%	1
GVTPYFVFL	0.00%	1	**SYRAYLKGY**	0.00%	1
HASRLVSTS	0.00%	1	**TPYFVFLKD**	4.77%	2
IAANPSGEE	0.00%	1	**TSFDKKSYR**	0.00%	1
IDLVHASRL	73.11%	12	**VALMNFREN**	4.77%	1
IFESRINEY	48.32%	9	**VFKDAISGD**	17.84%	1
INDVVYEVD	0.00%	2	**VFLKDGLIE**	0.00%	1
INEYMVNVF	42.50%	8	**VHASRLVST**	18.15%	7
ISGDEMFSD	20.57%	3	**VIDLVHASR**	23.19%	4
KAIKERLQK	0.00%	1	**VSDSTERVI**	18.23%	1
KGYLKAIKE	4.77%	1	**VSIFESRIN**	45.87%	7
KKSYRAYLK	28.63%	4	**VSNGLDSKL**	6.40%	1
KLIAANPSG	15.70%	2	**VTPYFVFLK**	11.53%	4
LDSKLIAAN	2.33%	2	**VVYEVDANF**	53.48%	9

LIAANPSGE	38.83%	5	**VYEVDANFI**	55.60%	10
LINDVVYEV	36.09%	8	**YEHYIGESM**	36.19%	6
LKAIKERLQ	33.26%	6	**YFVFLKDGL**	35.78%	8
LKDGLIEEK	0.00%	1	**YIGESMNPD**	28.85%	9
LKGYLKAIK	32.98%	5	**YLKAIKERL**	78.93%	16
LMNFRENGV	34.42%	6	**YLKGYLKAI**	46.65%	11
LQKENPERV	39.10%	5	**YMVNVFKNF**	11.30%	7
LVHASRLVS	29.35%	4	**YRAYLKGYL**	37.64%	8
Epitope set	**81.94%**				

Table 8. Population coverage of proposed MHC I epitopes in the world, northeast Asia and Southeast Asia.

Population / Area	Class I epitope	**YMVNVFKNF**	**VVYEVDANF**	**YEHYIGESM**	**FRENGVTPY**
	Total HLA hits	7	8	7	7
	Epitope set coverage	**Epitope coverage**			
World	**80.49%**	**31.13%**	**37.10%**	**41.07%**	**64.07%**
Northeast Asia	**77.88%**	**39.33%**	**47.10%**	**43.87%**	**55.03%**
China	76.68%	38.69%	45.78%	42.77%	54.46%
Southeast Asia	**80.37%**	**24.52%**	**37.26%**	**44.85%**	**53.45%**
Indonesia	52.02%	27.73%	37.53%	19.66%	22.89%
Philippines	90.80%	43.93%	43.93%	65.31%	57.07%

Table 9. Population coverage of proposed MHC II epitopes in the world, northeast Asia and Southeast Asia.

Population / Area	Class II epitope	**IDLVHASRL**	**YLKAIKERL**	**YLKGYLKAI**
	Total HLA hits	12	16	11
	Epitope set coverage	**Epitope coverage**		
World	**81.94%**	**73.11%**	**78.93%**	**46.65%**
Northeast Asia	**56.89%**	**53.44%**	**54.58%**	**28.07%**
China	56.89%	53.44%	54.58%	28.07%
Southeast Asia	**56.13%**	**53.56%**	**54.66%**	**24.75%**
Indonesia	45.91%	45.28%	44.43%	25.95%
Philippines	27.53%	27.53%	27.53%	5.89%

Allergenicity test:

Allergenicity of both B cell and T cell selected epitopes were tested using AllerTopv.2.0 software to avoid production of IgE antibodies as possible. Results of this test are listed in **Table 10.**

Table 10. Result of Allergenicity Test of predicted B cell and MHC class I & II epitopes.

B cell epitopes	Result	MHC I epitopes	Result	MHC II epitopes	Result
DYEHYI	Probable non-alleregen	**YMVNVFKNF**	Probable non-alleregen	**IDLVHASRL**	Probable non-alleregen
DYEHY	Probable alleregen	**VVYEVDANF**	Probable alleregen	**YLKAIKERL**	Probable non-alleregen
YEHYI	Probable non-alleregen	**YEHYIGESM**	Probable non-alleregen	**YLKGYLKAI**	Probable non-alleregen
		FRENGVTPY	Probable non-alleregen		

Discussion

There is an urgent need for developing a vaccine as a major aspect to control schistosomiasis as is still making significant public health concern in their endemic countries even with efforts done to control it (20, 63, 64). Therefore, in the current study, we have selected *TCTP* as a target for vaccine design using in silico study, as it has many advantages over traditional peptide vaccine development methods (65-67). Additionally, this in silico approach was used to design peptide vaccine against several diseases and numerous promising peptides vaccine were investigated (68, 69). Bioinformatics analysis of *TCTP* from *Spirometra mansoni* which has the closest evolutionary position with *Schistosoma japonicum*, and *Schistosoma mansoni* revealed that it may be used as a potential vaccine candidate (70). Moreover, promising peptides were predicted from *Madurella mycetomatis TCTP* using an immmunoinformatics approach (71). Besides, the potential role of *TCTP* as a vaccine was investigated by MacDonald SM et al. in 2001 using *Plasmodium falciparum TCTP*. Accordingly, this protein can stimulate histamine release from basophils and

IL-8 secretion from eosinophils in vitro and may affect host immune responses in vivo. Additionally, according to Taylor et al. in 2015 *TCTP* from different Plasmodium species resulted in a significant reduction in parasitemia in the early stage of infection in BALB/c mice (72, 73). A set of 22 *TCTP* sequences 'most of them were collected from China' were retrieved as FASTA format from NCBI for this study until 9th of December 2017 and conserved regions among them were identified. These strains had high degree of conservancy among them and promiscuous epitopes were selected to be effective antigen for both B cells and T cells.

As B cells play a critical role in adaptive immunity, *SjTCTP* was subjected to Bepipred linear epitope prediction, Emini surface accessibility, and Kolaskar and Tongaonkar antigenicity prediction methods in IEDB to determine their potential effect as B cell antigen. Accordingly, several conserved epitopes having the length ranged from 2 to 19 amino acids were predicted, but not all of them got scores above the threshold in both Emini surface accessibility, and Kolaskar and Tongaonkar antigenicity. However, when we decreased the length of the linear epitope ^{128}**DYEHYIGESMNPDG**^{141} from 14 amino acid to 6, and 5 amino acid, the antigenicity, and surface accessibility score were raised above the threshold values as follow: epitope **YEHYI** from129 to 133 was found to have one of the highest score in antigenicity (**1.086**) and good score in surface accessibility (**1.054**), followed by **DYEHYI** from 128 to 133 and then **DYEHY** from 128 to 132 which produced the highest score in surface accessibility (**2.511**). On the other hand, T cells recognize epitopes when they are presented by MHC class I and MHC class II. Therefore, binding of peptide and MHC is an important step in the prediction of T-cell epitopes (74). A total of 54 conserved peptides of TCTP were predicted to bind with MHC class I alleles. Four epitopes, **FRENGVTPY**, **YEHYIGESM**, **VVYEVDANF,** and **YMVNVFKNF** were found to bind with a large number of MHC I with high and intermediate affinity. Among four epitopes, epitope ^{26} **VVYEVDANF**^{34} had higher affinity to interact with eight alleles, while other epitopes had an affinity to interact with seven alleles of each. In the case of MHC II, a set of 96 conserved epitopes were predicted. Among them, the following epitopes: **YLKAIKERL**, **IDLVHASRL,** and **YLKGYLKAI** were detected to bind with a large number of MHC II alleles. Epitope ^{92} **YLKAIKERL**^{100} was found to interact with the highest number of alleles, sixteen alleles. Epitopes **IDLVHASRL** and **YLKGYLKAI** interacted with twelve and eleven alleles respectively. In addition to that, several overlaps between MHC class I and II were observed in T cell epitopes.

These overlaps might increase the possibility of epitopes presentation to T cells via binding to both MHC class I and II.

MHC are highly polymorphic genes, and different populations express different types of MHC alleles. Thus, calculation of population coverage is highly recommended, and the high value of population coverage is required in the development of a universal epitope-based vaccine (57, 74). In this study, the population coverage of all MHC I epitopes in the world is 99.27%, while for MHC II is 81.94% as some alleles were missed from IEDB database and hence did not include in the calculation of population coverage of all predicted MHC II epitopes. Regarding our proposed MHC I and MHC II epitopes, maximum coverage in the world of MHC I (**64.07%**) resulted from epitope **FRENGVTPY** rather than **VVYEVDANF** despite it bound to seven alleles while the later one bound to eight alleles. On the other hand, the maximum population coverage of MHC II (**78.93%**) resulted from **YLKAIKERL** that bound to the largest number of MHC II alleles. When we calculated the population coverage of the proposed MHC I and MHC II epitopes in China, Indonesia, and Philippines 'the place where *S. japonicum* is endemic' different coverage of each epitope resulted in each country as there was a difference in the frequency distribution of human leukocyte antigen (HLA) class I and II alleles in those countries (75, 76). In the case of MHC I Epitopes, **FRENGVTPY** is a most promising one in China with population coverage 54.46%, while epitopes **VVYEVDANF** with population coverage 37.53%, and **YEHYIGESM** with coverage 65.31% are the most promising epitopes in Indonesia and Philippines respectively. Whereas, in MHC II epitopes, **YLKAIKERL** with coverage 54.58%, **IDLVHASRL** with coverage 45.28% and both **IDLVHASRL** and **YLKAIKERL** with population coverage 27.53% for each are the most promising epitopes in China, Indonesia, and Philippines respectively.

Although vaccination is an efficient method to prevent the diseases, the potential risk of allergic reactions exists. These allergic reactions are mediated by the reaction of IgE antibody with a vaccine itself or the vaccine components (77-79). Thus, our predicted B and T cell epitopes were subjected to allergenicity test using AllerTopv.2.0. In the case of B cell, two epitopes **DYEHYI** and **YEHYI** have a probability to be a real epitope, while all of the predicted T cell epitopes except **VVYEVDANF** could also be real epitopes as all of them have a non-allergic effect. *Schistosoma japonicum* is still causing a serious problem in its endemic countries, and until now no vaccines have shown a good immunogenic response. Therefore, in this study, our immunoinformatics

analysis aided to design a potential immunogenic and safe peptide vaccine which may have a promising preventive ability to control *S. japonicum*. However, to prove the efficacy of the predicted epitopes, additional in vitro and in vivo studies are required along with this computational study.

Conclusion

Immunoinformatics approaches have enabled the ability to design vaccines avoiding the disadvantages of the conventional methods as they reduce the time and cost required and enhance the safety and efficacy of the predicted epitopes (33, 80-86). Several epitopes were predicted in this study to design a vaccine against *Schistosoma japonicum* using in silico prediction tools. Epitope **YEHYIGESM** is recommended for further in vitro and in vivo studies to confirm its efficacy as a peptide vaccine, as this epitope is shared between two classes of MHC alleles and overlapped with the most promising B cell epitope.

Acknowledgement

The authors are thankful to all members of Africa City of Technology for their help.

Conflict of Interest

Authors declare that there is no conflict of interest.

References

1. Hu W, Yan Q, Shen DK, Liu F, Zhu ZD, Song HD, et al. Evolutionary and biomedical implications of a Schistosoma japonicum complementary DNA resource. Nat Genet. 2003;35(2):139-47.
2. Steinmann P. Epidemiology and diagnosis of "Schistosoma japonicum", other helminth infections and multiparasitism in Yunnan province, People's Republic of China2008.
3. The Schistosoma japonicum Genome S, Functional Analysis C. The Schistosoma japonicum genome reveals features of host–parasite interplay. Nature. 2009;460:345.
4. Bartoníček Z. Calcium and calcium-dependent proteins in biology of schistosomes [BACHELOR THESIS]. PRAGUE: CHARLES UNIVERSITY 2016.
5. WHO. Schistosomiasis Fact sheet [updated March 2018]. Available from: http://www.who.int/mediacentre/factsheets/fs115/en/.
6. Inobaya MT, Olveda RM, Chau TNP, Olveda DU, Ross AGP. Prevention and control of schistosomiasis: a current perspective. Research and reports in tropical medicine. 2014;2014(5):65-75.
7. Chen MG. Assessment of morbidity due to Schistosoma japonicum infection in China. Infect Dis Poverty. 2014;3(1):6.

8. Shen J, Lai D-H, Wilson RA, Chen Y-F, Wang L-F, Yu Z-L, et al. Nitric oxide blocks the development of the human parasite Schistosoma japonicum. Proceedings of the National Academy of Sciences. 2017;114(38):10214-9.
9. Hong Y, Sun A, Zhang M, Gao F, Han Y, Fu Z, et al. Proteomics analysis of differentially expressed proteins in schistosomula and adult worms of Schistosoma japonicum. Acta Trop. 2013;126(1):1-10.
10. Barry MA, Simon GG, Mistry N, Hotez PJ. Global trends in neglected tropical disease control and elimination: impact on child health. Arch Dis Child. 2013;98(8):635-41.
11. Inobaya MT, Olveda RM, Chau TN, Olveda DU, Ross AG. Prevention and control of schistosomiasis: a current perspective. Research and reports in tropical medicine. 2014;2014(5):65.
12. Roure S, Valerio L, Pérez-Quílez O, Fernández-Rivas G, Martínez-Cuevas O, Alcántara-Román A, et al. Epidemiological, clinical, diagnostic and economic features of an immigrant population of chronic schistosomiasis sufferers with long-term residence in a non-endemic country (North Metropolitan area of Barcelona, 2002-2016). PLOS ONE. 2017;12(9):e0185245.
13. He YX, Salafsky B, Ramaswamy K. Host--parasite relationships of Schistosoma japonicum in mammalian hosts. Trends Parasitol. 2001;17(7):320-4.
14. Cao Z, Huang Y, Wang T. Schistosomiasis Japonica Control in Domestic Animals: Progress and Experiences in China. Frontiers in Microbiology. 2017;8(2464).
15. Cioli D, Pica-Mattoccia L, Basso A, Guidi A. Schistosomiasis control: praziquantel forever? Mol Biochem Parasitol. 2014;195(1):23-9.
16. Colley DG, Bustinduy AL, Secor WE, King CH. Human schistosomiasis. Lancet. 2014;383(9936):2253-64.
17. Gray DJ, McManus DP, Li Y, Williams GM, Bergquist R, Ross AG. Schistosomiasis elimination: lessons from the past guide the future. Lancet Infect Dis. 2010;10(10):733-6.
18. Ricciardi A, Ndao M. Still hope for schistosomiasis vaccine. Hum Vaccin Immunother. 2015;11(10):2504-8.
19. Siddiqui AA, Siddiqui BA, Ganley-Leal L. Schistosomiasis vaccines. Hum Vaccin. 2011;7(11):1192-7.
20. Li C, Yu L, Liu Z, Zhu L, Hu Y, Zhu M, et al. Schistosoma japonicum: the design and experimental evaluation of a multivalent DNA vaccine. Cell Mol Biol Lett. 2006;11(4):449-60.
21. McManus DP, Loukas A. Current status of vaccines for schistosomiasis. Clin Microbiol Rev. 2008;21(1):225-42.
22. Bergquist R, Utzinger J, Keiser J. Controlling schistosomiasis with praziquantel: How much longer without a viable alternative? Infectious Diseases of Poverty. 2017;6(1):74.
23. El Ridi R, Othman AA, McManus DP. Editorial: The Schistosomiasis Vaccine - It is Time to Stand up. Front Immunol. 2015;6:390.
24. J M, Lee K. Secretion of Translationally Controlled Tumor Protein/Histamine Releasing Factor and Its Relevance to Parasitic Infections. Pharmaceutica Analytica Acta. 2014;5:304.
25. Chen H, Nara T, Zeng X, Satoh M, Wu G, Jiang W, et al. Vaccination of domestic pig with recombinant paramyosin. against Schistosoma japonicum in China. Vaccine. 2000;18(20):2142-6.
26. Liu J, Wang J, Wang S, Xu B, Liu X, Wang X, et al. Molecular cloning and characterization of Schistosoma japonicum aldose reductase. Parasitol Res. 2013;112(2):549-58.
27. Qiu C, Liu S, Hong Y, Fu Z, Wei M, Ai D, et al. Molecular characterization of thyroid hormone receptor beta from Schistosoma japonicum and assessment of its potential as a vaccine candidate antigen against schistosomiasis in BALB/c mice. Parasites & vectors. 2012;5(1):172.
28. Tebeje BM, Harvie M, You H, Loukas A, McManus DP. Schistosomiasis vaccines: where do we stand? Parasit Vectors. 2016;9(1):528.
29. Wei F, Liu Q, Gao S, Shang L, Zhai Y, Men J, et al. Enhancement by IL-18 of the protective effect of a Schistosoma japonicum 26kDa GST plasmid DNA vaccine in mice. Vaccine. 2008;26(33):4145-9.
30. Bergquist NR, Colley DG. Schistosomiasis vaccine:research to development. Parasitol Today. 1998;14(3):99-104.

31. RUAN Z-y, WU Z-d, CAO A-l. Immuno-Responses of Genetic Immunization of BALB/c Mice with Recombinant Plasmid pEGFP Containing TCTP Gene of Schistosoma japonicum. Journal of Tropical Medicine. 2008;10:005.
32. Zhang L. Multi-epitope vaccines: a promising strategy against tumors and viral infections. Cellular &Amp; Molecular Immunology. 2017;15:182.
33. Skwarczynski M, Toth I. Peptide-based synthetic vaccines. Chemical Science. 2016;7(2):842-54.
34. Mirza MU, Rafique S, Ali A, Munir M, Ikram N, Manan A, et al. Towards peptide vaccines against Zika virus: Immunoinformatics combined with molecular dynamics simulations to predict antigenic epitopes of Zika viral proteins. Scientific Reports. 2016;6:37313.
35. Khan MA, Hossain MU, Rakib-Uz-Zaman SM, Morshed MN. Epitope-based peptide vaccine design and target site depiction against Ebola viruses: an immunoinformatics study. Scand J Immunol. 2015;82(1):25-34.
36. Lata KS, Kumar S, Vaghasia V, Sharma P, Bhairappanvar SB, Soni S, et al. Exploring Leptospiral proteomes to identify potential candidates for vaccine design against Leptospirosis using an immunoinformatics approach. Scientific Reports. 2018;8(1):6935.
37. Dellagostin OA, Grassmann AA, Rizzi C, Schuch RA, Jorge S, Oliveira TL, et al. Reverse Vaccinology: An Approach for Identifying Leptospiral Vaccine Candidates. International Journal of Molecular Sciences. 2017;18(1):158.
38. Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, et al. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. 2008;36(Web Server issue):19.
39. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series. 1999;41:95-8.
40. Hasan MA, Hossain M, Alam MJ. A Computational Assay to Design an Epitope-Based Peptide Vaccine Against Saint Louis Encephalitis Virus. Bioinform Biol Insights. 2013;7:347-55.
41. Potocnakova L, Bhide M, Pulzova LB. An Introduction to B-Cell Epitope Mapping and In Silico Epitope Prediction. Journal of Immunology Research. 2016;2016:11.
42. Vita R, Overton JA, Greenbaum JA, Ponomarenko J, Clark JD, Cantrell JR, et al. The immune epitope database (IEDB) 3.0. Nucleic Acids Research. 2015;43(D1):D405-D12.
43. Larsen JE, Lund O, Nielsen M. Improved method for predicting linear B-cell epitopes. Immunome Res. 2006;2(2):1745-7580.
44. Emini EA, Hughes JV, Perlow DS, Boger J. Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide. J Virol. 1985;55(3):836-9.
45. Kolaskar AS, Tongaonkar PC. A semi-empirical method for prediction of antigenic determinants on protein antigens. FEBS Lett. 1990;276(1-2):172-4.
46. Ahmad T, Ewida A, El-Sayed L. T-cell epitope mapping for the design of powerful vaccines2016. 13-22 p.
47. Kringelum JV, Nielsen M, Padkjaer SB, Lund O. Structural analysis of B-cell epitopes in antibody:protein complexes. Mol Immunol. 2013;53(1-2):24-34.
48. Patronov A, Doytchinova I. T-cell epitope vaccine design by immunoinformatics. Open Biol. 2013;3(1):120139.
49. Nielsen M, Lundegaard C, Worning P, Lauemoller SL, Lamberth K, Buus S, et al. Reliable prediction of T-cell epitopes using neural networks with novel sequence representations. Protein Sci. 2003;12(5):1007-17.
50. Fleri W, Paul S, Dhanda SK, Mahajan S, Xu X, Peters B, et al. The Immune Epitope Database and Analysis Resource in Epitope Discovery and Synthetic Vaccine Design. Frontiers in Immunology. 2017;8(278).
51. Sette A, Vitiello A, Reherman B, Fowler P, Nayersina R, Kast WM, et al. The relationship between class I binding affinity and immunogenicity of potential cytotoxic T cell epitopes. J Immunol. 1994;153(12):5586-92.

52. Greenbaum J, Sidney J, Chung J, Brander C, Peters B, Sette A. Functional classification of class II human leukocyte antigen (HLA) molecules reveals seven different supertypes and a surprising degree of repertoire sharing across supertypes. Immunogenetics. 2011;63(6):325-35.
53. Wang P, Sidney J, Dow C, Mothé B, Sette A, Peters B. A Systematic Assessment of MHC Class II Peptide Binding Predictions and Evaluation of a Consensus Approach. PLoS Comput Biol. 2008;4(4):1000048.
54. Li Z, Zhao Y, Pan G, Tang J, Guo F. A Novel Peptide Binding Prediction Approach for HLA-DR Molecule Based on Sequence and Structural Information. BioMed Research International. 2016;2016:10.
55. Nielsen M, Lund O. NN-align. An artificial neural network-based alignment algorithm for MHC class II peptide binding prediction. BMC Bioinformatics. 2009;10:296.
56. Sakib MS, Islam MR, Hasan AKMM, Nabi AHMN. Prediction of Epitope-Based Peptides for the Utility of Vaccine Development from Fusion and Glycoprotein of Nipah Virus Using In Silico Approach. Advances in Bioinformatics. 2014;2014:17.
57. Bui HH, Sidney J, Dinh K, Southwood S, Newman MJ, Sette A. Predicting population coverage of T-cell epitope-based diagnostics and vaccines. BMC Bioinformatics. 2006;7(153):1471-2105.
58. Dimitrov I, Bangov I, Flower DR, Doytchinova I. AllerTOP v.2--a server for in silico prediction of allergens. J Mol Model. 2014;20(6):014-2278.
59. Källberg M, Wang H, Wang S, Peng J, Wang Z, Lu H, et al. Template-based protein structure modeling using the RaptorX web server. Nature Protocols. 2012;7:1511.
60. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem. 2004;25(13):1605-12.
61. Ayub G, Waheed Y, Najmi MH. Prediction and conservancy analysis of promiscuous T-cell binding epitopes of Ebola virus L protein: An in silico approach. Asian Pacific Journal of Tropical Disease. 2016;6(3):169-73.
62. Bui HH, Sidney J, Li W, Fusseder N, Sette A. Development of an epitope conservancy analysis tool to facilitate the design of epitope-based diagnostics and vaccines. BMC Bioinformatics. 2007;8(361):1471-2105.
63. Cao Y, Qiao H, Shi Y, Han Y, Liu J, Li H, et al. Evaluation of Lethal Giant Larvae as a Schistosomiasis Vaccine Candidate. BioMed Research International. 2016;2016:7.
64. Molehin AJ, Sennoune SR, Zhang W, Rojo JU, Siddiqui AJ, Herrera KA, et al. Cross-species prophylactic efficacy of Sm-p80-based vaccine and intracellular localization of Sm-p80/Sm-p80 ortholog proteins during development in Schistosoma mansoni, Schistosoma japonicum, and Schistosoma haematobium. Parasitol Res. 2017;116(11):3175-88.
65. Chen H, Yang HW, Wei JF, Tao AL. In silico prediction of the T-cell and IgE-binding epitopes of Per a 6 and Bla g 6 allergens in cockroaches. Mol Med Rep. 2014;10(4):2130-6.
66. Li W, Joshi MD, Singhania S, Ramsey KH, Murthy AK. Peptide Vaccine: Progress and Challenges. Vaccines (Basel). 2014;2(3):515-36.
67. Arnon R, Tarrab-Hazdai R, Steward M. A mimotope peptide-based vaccine against Schistosoma mansoni: synthesis and characterization. Immunology. 2000;101(4):555-62.
68. Ahmed Hamdi Abu-haraz KAA-e, Mojahid Salah Ibrahim, Waleed Hassan Hussien, Mohammed Siddig Mohammed, Marwan Mustafa Badawi and Mohamed Ahmed Salih. Multi Epitope Peptide Vaccine Prediction against Sudan Ebola Virus Using Immuno-Informatics Approaches Advanced Techniques in Biology & Medicine. 2017;5:203.
69. Badawi MM, Osman MM, Alla AAF, Ahmedani AM, Abdalla Mh, Gasemelseed MM, et al. Highly Conserved Epitopes of ZIKA Envelope Glycoprotein May Act as a Novel Peptide Vaccine with High Coverage: Immunoinformatics Approach. American Journal of Biomedical Research. 2016;4(3):46-60.
70. Lu YJ, Lu G, Shi DZ, Li LH, Zhong SF. Bioinformatic analysis for structure and function of TCTP from Spirometra mansoni. Asian Pac J Trop Med. 2013;6(9):709-12.
71. Samira Bolis WO, Mohamed Anwar, Masajed Abdelmagid, Esameldeen Ahmed, Mohammed Abaker, Wiaam Abdelwahab, Suzan Hashim, Aisha Abubakralsiddig, Abeer Algaali, Omar Hashim, and

Mohamed Hassan. Immunoinformatics Prediction of Epitope Based Peptide Vaccine Against Madurella mycetomatis Translationally Controlled Tumor Protein. bioRxiv. 2018.
72. MacDonald SM, Bhisutthibhan J, Shapiro TA, Rogerson SJ, Taylor TE, Tembo M, et al. Immune mimicry in malaria: Plasmodium falciparum secretes a functional histamine-releasing factor homolog in vitro and in vivo. Proc Natl Acad Sci U S A. 2001;98(19):10829-32.
73. Taylor KJ, Van TT, MacDonald SM, Meshnick SR, Fernley RT, Macreadie IG, et al. Immunization of mice with Plasmodium TCTP delays establishment of Plasmodium infection. Parasite Immunol. 2015;37(1):23-31.
74. Sanchez-Trincado JL, Gomez-Perosanz M, Reche PA. Fundamentals and Methods for T- and B-Cell Epitope Prediction. J Immunol Res. 2017;2017:2680160.
75. Vina MAF, Hollenbach JA, Lyke KE, Sztein MB, Maiers M, Klitz W, et al. Tracking human migrations by the analysis of the distribution of HLA alleles, lineages and haplotypes in closed and open populations. Philosophical Transactions of the Royal Society B: Biological Sciences. 2012;367(1590):820-9.
76. Shankarkumar U. Complexities and similarities of HLA antigen distribution in Asian subcontinent. Indian Journal of Human Genetics. 2010;16(3):108-10.
77. Gershwin LJ. Adverse Reactions to Vaccination: From Anaphylaxis to Autoimmunity. Vet Clin North Am Small Anim Pract. 2018;48(2):279-90.
78. Dreskin SC, Halsey NA, Kelso JM, Wood RA, Hummell DS, Edwards KM, et al. International Consensus (ICON): allergic reactions to vaccines. World Allergy Organization Journal. 2016;9(1):32.
79. Chung EH. Vaccine allergies. Clinical and Experimental Vaccine Research. 2014;3(1):50-7.
80. Yasmin T, Akter S, Debnath M, Ebihara A, Nakagawa T, Nabi AHMN. In silico proposition to predict cluster of B- and T-cell epitopes for the usefulness of vaccine design from invasive, virulent and membrane associated proteins of C. jejuni. In Silico Pharmacology. 2016;4:5.
81. Srivastava PN, Jain R, Dubey SD, Bhatnagar S, Ahmad N. Prediction of Epitope-Based Peptides for Vaccine Development from Coat Proteins GP2 and VP24 of Ebola Virus Using Immunoinformatics. International Journal of Peptide Research and Therapeutics. 2016;22(1):119-33.
82. Tomar N, De RK. Immunoinformatics: an integrated scenario. Immunology. 2010;131(2):153-68.
83. von Herrath M, Taylor P. Immunoinformatics: an overview of computational tools and techniques for understanding immune function. Expert Rev Clin Immunol. 2007;3(6):993-1002.
84. Tomar N, De RK. Immunoinformatics: a brief review. Methods Mol Biol. 2014;1184:23-55.
85. Soria-Guerra RE, Nieto-Gomez R, Govea-Alonso DO, Rosales-Mendoza S. An overview of bioinformatics tools for epitope prediction: implications on vaccine development. J Biomed Inform. 2015;53:405-14.
86. Ali M, Pandey RK, Khatoon N, Narula A, Mishra A, Prajapati VK. Exploring dengue genome to construct a multi-epitope based subunit vaccine by utilizing immunoinformatics approach to battle against dengue infection. Scientific Reports. 2017;7(1):9232.