Epitope characterization and docking studies on Chikungunya viral Envelope 2 protein

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***Abstract*-** Pathogenic aspects of Chikungunya virus requires detailed study in order to develop drugs for controlling the outspread of Chikungunya infection. Previously it has been identified that Chikungunya viral envelope 1 and 2 proteins (E1 and E2) and the nonstructural protein 2 (nsP2) are involved in CHIKV pathogenesis. In this study, a reverse vaccinology approach has been used to elucidate the epitopic peptides associated with the envelope protein E2 of CHIKV. The study characterizes as well as maps B cell and T cell epitopes of the protein using various bioinformatics tools. Further, the predicted epitopes were modeled and docked with human receptors (2X40 and 1DLH) to analyze the binding affinities. The epitopes with high binding affinities for human receptors were identified as effective epitopes. We anticipate that the peptides identified as most effective epitopes from this study can be considered for designing epitope-based vaccines against Chikungunya disease.

***Index Terms*-** Chikungunya Virus, Envelope 2 protein, T cell & B cell Epitopes, Epitope modeling, Docking Studies, Binding Affinity

I. INTRODUCTION

Chikungunya is a widespread infectious disease with unpredictable emerging and reemerging disease outbreaks [1]. High fever and joint pain are the common symptoms of the disease, and other symptoms include vomiting, rashes, arthralgia, nausea and swelling of joints [2]. The disease is mainly endemic in Asia, Africa and Indian Ocean Islands. Previous studies have classified Chikungunya viral strains into three major genotypes, Asian, West African and East Central South African (ECSA) [3]. Mosquitoes of *Aedes* genus which include *Aedes aegypti*, *Aedes albopictus*, *Aedes africanus Aedes furcifer*, *Aedes taylori*, and *Aedes luteocephalus* has been well documented as a vector to the pathogen [3]. CHIKV genome is approximately 11.8Kb in length, consisting of two ORFs, coding for structural and non-structural proteins [4]. The 5'end ORF encode structural polyprotein, which later cleaves into four non-structural proteins (nsP1-nsP4) endowed with enzymatic properties. CHIKV nsP1 protein functions as an antagonist for the bone marrow stromal antigen 2, a host defense mechanism and down regulate BST-2 expression [5]. nsP1 also partakes in cytoplasmic capping, where7-methyl-GMP is covalently bonded to nsP1 to form the m7GMp-nsP1 complex, which is transferred to the mRNA to form the cap structure. nsP2 protein has two domains, N terminal and C terminal domain. The N terminal is found to have different enzymatic activities, the RNA triphospatase activity plays a key role in initiating the viral RNA capping reactions, the nucleotide triphospatase activity triggers the RNA helicase activity of nsP2 C-terminal domain, and the 5'-triphosphatase activity eliminates the ˊY-phosphate from the 5'end of the RNA. The protease activity of the nsP2, C terminal domain cleaves the polyprotein into four nonstructural proteins. nsP2 protein is involved in transcriptional and translational shutoff in infected host cells. (Not required) Recent studies have proved that Chikungunya viral nsP2 has the ability to block JAK-STAT signaling that inhibits viral replication [6]. The nsP3 protein is involved in 26S mRNA synthesis [7]. CHIKV nsP4 protein participates in genome replication, the protein replicates genomic and antigenomic RNA in addition to that nsP4 transcribes a 26S subgenomic mRNA, which codes for structural proteins. The CHIKV structural poly protein cleaves into E1, E2 and E3 envelope glycoproteins, 6k and capsid protein. The envelop protein E1 and E2 are antigenic proteins and enable the viral entry into the host cell. E1 protein is involved in membrane fusion and E2 protein is associated with receptor binding. The genomic organization of Chikungunya virus is as follows; 5'cap-nsP1-nsP2-nsP3-nsP4-(junction region)-C- E3-E2-6K-E1-poly(A) 3'[8].

Antigenic aspects of Chikungunya proteins need to be further explored to develop specific drugs for Chikungunya infection. The antigenicity associated with Chikungunya viral proteins are only poorly studied, however E2, E1 and nsP2 proteins were found to have antigenic properties [9]. The only available treatment of the disease is symptomatic treatment, an effective medicine for the disease has not been developed yet [10]. Antigenic analysis and *in silico* epitope prediction in CHIKV proteins can explore antigenic elements involved in Chikungunya infection. An understanding of the antigenic epitopes is essential for designing peptide-based vaccines [11]. Epitopes are part of the antigen in the pathogen that evokes immune responses in host organisms. Epitopes that interacts with B cell receptors are termed as B cell epitopes and those interact with T cell receptors are called T cell epitopes. B cell epitopes are of two types, Linear (continuous) epitopes and conformational (discontinuous) epitope [12]. T cell epitopes are the antigenic parts that induce immune responses when recognized by T cell lymphocytes [13]. *In vitro* screening of epitopes is time consuming and at the same time expensive too. *In silico* reverse vaccinolgy approaches can be used to study the antigenic elements in the pathogen [14]. Many immunoinformatics tools are available for analyzing antigenic

properties and epitopes associated with the pathogen. ANTIGENpro, BCPred, SVMTriP, COBEPro and IEDB tools like BepiPred, Ellipro etc are the commonly used immune informatics tools [15-17].

In the present investigation, we have identified B cell and T cell epitopes associated with Chikungunya viral antigenic protein, E2. Epitope mapping was carried out, in order to locate these identified epitopes. The epitopes were modeled and a docking study was performed on these epitopes with human receptors to study the binding affinities of the predicted CHIKV epitopes. The epitopic peptides with higher affinities towards the human receptors were identified as most efficient epitopes that can be considered for epitope based vaccine design.

II. MATERIALS AND METHODS

B cell epitope prediction

The CHIKV E2 protein sequence (Q0JRL8) was retrieved from Uniprot in FASTA format. The possible linear and conformation B cell epitopes of CHIKV E2 protein was identified using various Bioinformatics tools. B cell linear epitopes were predicted using the tools BepiPred ,BCPred, SVMTriP, COBEPro and Ellipro which embeds specific algorithm in epitope prediction (15-20).

T cell Epitope prediction

Identification of T cell epitopes is an important step in analyzing disease pathogenesis. In order to predict the T cell Epitopes, the CHIKV E2 (Q0JRL80) protein sequence was given to T cell Epitope prediction tools; IEDB MHC I & II Binding Prediction tool. Different programs have incorporated in IEDB's T cell epitope prediction tools to predict T cell epitopes. MHC I Binding prediction tool has programs such as Consensus, NetMHCpan, ANN, SMM, SMMPMBEC and CombLib whereas in MHC II Binding Prediction tool, in addition to the above mentioned programs, this also include methods like NN_align, SMM_align, and sturniolo. [21-22].

Epitope Modelling

For further analyzing the predicted epitopes, three-dimensional structures of the epitopes are required. The three-dimensional structures of these predicted CHIKV E2 epitopes were modeled using Pepstr, an effective web server for predicting the three dimensional structures of small peptides [23-24]

Docking

Epitope receptor docking studies were carried out using the docking server, PatchDOCK and further refined the docking results with FireDock [25-28]. The human receptors and the epitopes were imported to patch dock server. The epitopes of MHC I alleles were docked with human receptor 2X40 whereas the MHC II alleles were docked with 1DLH human receptor. Clustering RMSD was given as 4.0 Å, and then the docking jobs were submitted to the Patchdock server. Followed by this the rigid-body protein-protein docking solutions obtained from the PatchDock server were further refined by FireDock, which renders addition refinement to the docking complexes. Fire dock server analyses and sorts the refined complex structures based on energy functions like global energy atomic contact energy, contribution of the hydrogen bonds (HB) van der Waals interactions etc. Lower global energy indicates higher binding affinities with human receptors, the epitopic peptides that were having higher binding affinities were identified as most effective epitopes.

III. RESULTS

B cell epitope prediction of CHIKV E2 protein (Q0JRL8)

The details of B cell linear epitopes of CHIKV E2 protein (Q0JRL8) predicted using five linear B cell epitope prediction tools, Bepipred, BCPred, SVMtriP, COBEpro, and Ellipro were given in Supplementary Material 1. A graphical representation of B cell linear epitopes of E2 protein is shown in Figure 1.

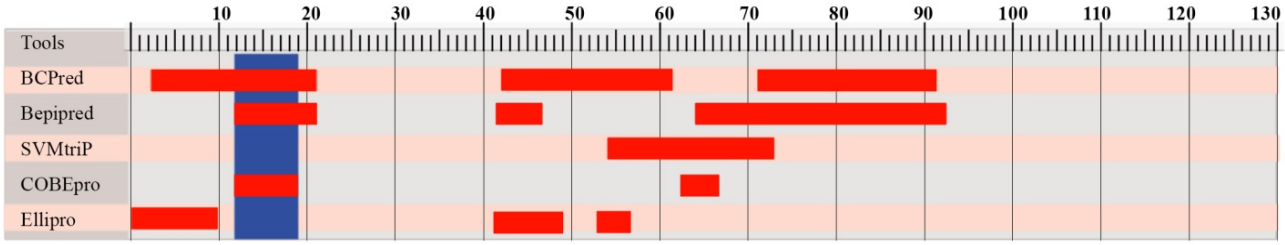

Figure 1: Graphical representation of B cell linear epitopes of CHIKV E2 protein (Q0JRL8) (Give a detailed mention of the picture)

We have identified the most probable epitope by analyzing the results obtained from all the linear B cell epitope prediction tool. The most probable B cell Liner Epitope of E2 glyco protein identified from this study is 'ARNPTV'; Sequence position 12-19. B cell Discontinuous Epitopes of CHIKV E2protein (Q0JRL8) were predicted using the tool Ellipro. The details of the discontinuous epitopes predicted by Ellipro are shown in Table 1.

Table 1: Details of Discontinuous epitopes of CHIKV E2 protein (Q0JRL8) obtained with Ellipro

No	Residues	Number of Residues	Score
1	_:L4, _:A5, _:N6	3	0.800

Epitope Mapping

Epitope mapping of B cell Liner Epitope of E2 glycoprotein is shown in Figure 2. The space-filled balls indicate the predicted epitope.

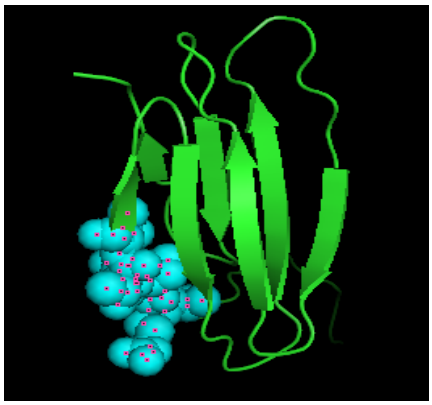

Figure 2: Epitope mapping of B cell Liner Epitope of E2 glycoprotein (please discuss about the picture)

T cell epitopes

The T cell epitope prediction was performed on human MHC class I & Class II alleles. The predicted T cell epitopes of CHIKV E2 protein (Q0JRL8) are given in Table 2 & 3 respectively.

Table 2: Details of MHC-I predicted T cell epitopes of CHIKV E2 protein (Q0JRL8)

Allele	Position	Peptide
HLA-A*01:01	71-79	VTWGNNEPY
HLA-A*02:01	119-127	FILLSMVGV
HLA-A*02:06	119-127	FILLSMVGV
HLA-A*03:01	13-21	KARNPTVTY
HLA-A*11:01	115-123	SVASFILLS
HLA-A*23:01	101-109	YYYELYPTM
HLA-A*24:02	101-109	YYYELYPTM
HLA-A*25:01	97-105	EIILYYYEL
HLA-A*26:01	94-102	HPHEIILYY
HLA-A*29:02	98-106	IILYYYELY
HLA-A*30:01	13-21	KARNPTVTY
HLA-A*30:02	23-31	KNQVIMLLY
HLA-A*31:01	7-15	VTCRVPKAR
HLA-A*32:01	19-27	VTYGKNQVI
HLA-A*68:01	53-61	WVTHKKEIR
HLA-A*68:02	109-117	MTVVVVSVA
HLA-B*07:02	106-114	YPTMTVVVV
HLA-B*08:01	11-19	VPKARNPTV
HLA-B*14:02	116-124	VASFILLSM

Table 3: MHC-II predicted T cell epitopes of CHIKV E2 protein (Q0JRL8)

Allele	Position	Peptide	Percentile rank
HLA-DRB1*01:01	100-114	LYYYELYPTMTVVVV	2.27
HLA-DRB1*01:02	107-121	PTMTVVVVSVASFIL	0.44
HLA-DRB1*03:01	106-120	YPTMTVVVVSVASFI	0.36
HLA-HLA-DRB1*03:05	114-128	VSVASFILLSMVGVA	0.77
HLA-HLA-DRB1*03:06	24-38	NQVIMLLYPDHPTLL	1.24
HLA-HLA-DRB1*03:07	24-38	NQVIMLLYPDHPTLL	1.24
HLA-HLA-DRB1*03:08	24-38	NQVIMLLYPDHPTLL	1.24
HLA-HLA-DRB1*03:09	47-61	PNYQEEWVTHKKEIR	0.81
HLA-HLA-DRB1*04:01	98-112	IILYYYELYPTMTVV	1.29
HLA-HLA-DRB1*04:02	13-27	KARNPTVTYGKNQVI	2.15
HLA-HLA-DRB1*04:04	24-38	NQVIMLLYPDHPTLL	0.14
HLA-HLA-DRB1*04:05	98-112	IILYYYELYPTMTVV	1.75
HLA-HLA-DRB1*04:08	97-111	EIILYYYELYPTMTV	0.89
HLA-HLA-DRB1*04:10	31-45	YPDHPTLLSYRNMGE	0.63
HLA-HLA-DRB1*04:21	54-68	VTHKKEIRLTVPTEG	1.39
HLA-HLA-DRB1*04:23	105-119	LYPTMTVVVVSVASF	0.84
HLA-HLA-DRB1*04:26	25-39	QVIMLLYPDHPTLLS	1.33
HLA-HLA-DRB1*07:01	108-122	TMTVVVVSVASFILL	0.09
HLA-HLA-DRB1*07:03	107-121	PTMTVVVVSVASFIL	0.04
HLA-HLA-DRB1*08:01	97-111	EIILYYYELYPTMTV	0.79
HLA-HLA-DRB1*08:02	105-119	LYPTMTVVVVSVASF	1.30
HLA-HLA-DRB1*08:04	105-119	LYPTMTVVVVSVASF	0.74
HLA-HLA-DRB1*08:06	105-119	LYPTMTVVVVSVASF	0.69
HLA-HLA-DRB1*08:13	97-111	EIILYYYELYPTMTV	0.30
HLA-HLA-DRB1*08:17	97-111	EIILYYYELYPTMTV	0.36
HLA-HLA-DRB1*09:01	100-114	LYYYELYPTMTVVVV	0.20
HLA-HLA-DRB1*11:01	73-87	WGNNEPYKYWPQLST	2.21
HLA-HLA-DRB1*11:02	47-61	PNYQEEWVTHKKEIR	2.80
HLA-HLA-DRB1*11:04	105-119	LYPTMTVVVVSVASF	0.84
HLA-HLA-DRB1*11:06	105-119	LYPTMTVVVVSVASF	0.84
HLA-HLA-DRB1*11:07	114-128	VSVASFILLSMVGVA	0.34
HLA-HLA-DRB1*11:14	47-61	PNYQEEWVTHKKEIR	0.52
HLA-HLA-DRB1*11:20	47-61	PNYQEEWVTHKKEIR	0.14
HLA-HLA-DRB1*11:21	47-61	PNYQEEWVTHKKEIR	2.80
HLA-HLA-DRB1*11:28	105-119	LYPTMTVVVVSVASF	0.37
HLA-HLA-DRB1*12:01	100-114	LYYYELYPTMTVVVV	10.03
HLA-HLA-DRB1*13:01	105-119	LYPTMTVVVVSVASF	0.71
HLA-HLA-DRB1*13:02	108-122	TMTVVVVSVASFILL	1.57
HLA-HLA-DRB1*13:04	105-119	LYPTMTVVVVSVASF	2.60
HLA-HLA-DRB1*13:05	105-119	LYPTMTVVVVSVASF	0.37
HLA-HLA-DRB1*13:07	105-119	LYPTMTVVVVSVASF	0.52
HLA-HLA-DRB1*13:11	105-119	LYPTMTVVVVSVASF	0.84
HLA-HLA-DRB1*13:21	105-119	LYPTMTVVVVSVASF	1.37
HLA-HLA-DRB1*13:22	47-61	PNYQEEWVTHKKEIR	2.80
HLA-HLA-DRB1*13:23	47-61	PNYQEEWVTHKKEIR	0.52
HLA-HLA-DRB1*13:27	105-119	LYPTMTVVVVSVASF	0.71
HLA-HLA-DRB1*13:28	105-119	LYPTMTVVVVSVASF	0.71
HLA-HLA-DRB1*15:01	110-124	TVVVVSVASFILLSM	0.65
HLA-HLA-DRB1*15:02	97-111	EIILYYYELYPTMTV	0.11
HLA-HLA-DRB1*15:06	107-121	PTMTVVVVSVASFIL	0.37
HLA-HLA-DRB3*01:01	24-38	NQVIMLLYPDHPTLL	0.01
HLA-HLA-DRB4*01:01	23-37	KNQVIMLLYPDHPTL	3.33
HLA-HLA-DRB5*01:01	110-124	TVVVVSVASFILLSM	1.28
HLA-HLA-DRB5*01:05	107-121	PTMTVVVVSVASFIL	0.26

Epitope Modeling of MHC epitopes: The modeled three-dimensional structures of CHIKV E2 MHC I & MHC II epitopes were modeled using Pepstr server. As a sample, modeled structure of CHIKV E2 MHC I HLA-A*02:06 epitope using Pepstr is shown in Figure 3.

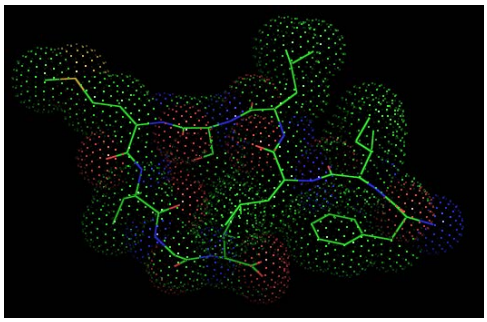

Figure 3: The modeled three-dimensional structures of CHIKV E2 MHC I HLA-A*02:06 epitope

Docking

We have selected the highest ranked candidate from FireDock, which has the lowest global energy, for each of the docking analysis. The details of docking analysis performed with MHC I & II epitopes were displayed in Table 4 and 5 respectively. Here, the global energy indicates binding affinity, lower the global energy higher will be its binding affinity. The epitopic petides with lower global energies with human receptors were identified as the most effective epitopes.

Table 4: The details of docking studies of CHIKV MHC I epitopes of E2 protein (Q0JRL8)

Epitope	Allele	Receptor	Global energy	Attractive VdW	Repulsive VdW	ACE	HB
VTWGNNEPY	HLA-A*01:01	2X40.pdb	-21.13	-26.13	27.99	-0.94	-3.13
FILLSMVGV	HLA-A*02:01	2X40.pdb	-13.79	-18.36	6.93	-2.68	-0.99
FILLSMVGV	HLA-A*02:06	2X40.pdb	-13.79	-18.36	6.93	-2.68	-0.99
KARNPTVTY	HLA-A*03:01	2X40.pdb	-26.10	-16.31	4.82	-5.09	0.00
SVASFILLS	HLA-A*11:01	2X40.pdb	-62.45	-30.31	21.00	-15.55	-1.99
YYYELYPTM	HLA-A*23:01	2X40.pdb	-45.89	-26.51	5.50	-7.96	-0.76
YYYELYPTM	HLA-A*24:02	2X40.pdb	-45.89	-26.51	5.50	-7.96	-0.76
EIILYYYEL	HLA-A*25:01	2X40.pdb	-30.75	-15.97	8.80	-9.86	0.00
HPHEIILYY	HLA-A*26:01	2X40.pdb	-51.94	-26.43	6.63	-9.21	-1.35
IILYYYELY	HLA-A*29:02	2X40.pdb	-52.00	-23.60	23.27	-13.35	-2.25
KARNPTVTY	HLA-A*30:01	2X40.pdb	-26.10	-16.31	4.82	-5.09	0.00
KNQVIMLLY	HLA-A*30:02	2X40.pdb	-3.99	-27.19	51.27	-9.08	-1.69
VTCRVPKAR	HLA-A*31:01	2X40.pdb	-17.00	-19.25	16.21	-2.13	-1.00
VTYGKNQVI	HLA-A*32:01	2X40.pdb	-19.89	-20.13	7.28	-1.78	-0.34
WVTHKKEIR	HLA-A*68:01	2X40.pdb	-44.36	-19.21	2.93	-6.26	0.00
MTVVVVSVA	HLA-A*68:02	2X40.pdb	-33.29	-25.37	14.58	-8.38	-2.40
YPTMTVVVV	HLA-B*07:02	2X40.pdb	-62.44	-29.51	14.22	-13.47	-2.03

VPKARNPTV	HLA-B*08:01	2X40.pdb	-25.54	-25.44	14.94	-1.74	-0.32
VASFILLSM	HLA-B*14:02	2X40.pdb	-33.11	-17.35	8.86	-6.24	0.00

Table 5: The details of docking studies of CHIKV MHC II epitopes of E2 protein (Q0JRL8)

Peptide	Allele	Receptor	Global energy	Attractive VdW	Repulsive VdW	ACE	HB
LYYYELYPTMTVVVV	HLA-DRB1*01:01	1DLH.pdb	-84.98	-36.04	31.40	-16.14	-2.53
PTMTVVVVSVASFIL	HLA-DRB1*01:02	1DLH.pdb	-85.20	-26.31	17.95	-19.39	-0.86
YPTMTVVVVSVASFI	HLA-DRB1*03:01	1DLH.pdb	-70.05	-24.65	14.82	-15.32	-0.38
VSVASFILLSMVGVA	HLA-HLA-DRB1*03:05	1DLH.pdb	-60.96	-26.34	8.82	-7.85	-1.42
NQVIMLLYPDHPTLL	HLA-HLA-DRB1*03:06	1DLH.pdb	-51.03	-33.77	38.54	-10.26	-0.40
NQVIMLLYPDHPTLL	HLA-HLA-DRB1*03:07	1DLH.pdb	-51.03	-33.77	38.54	-10.26	-0.40
NQVIMLLYPDHPTLL	HLA-HLA-DRB1*03:08	1DLH.pdb	-51.03	-33.77	38.54	-10.26	-0.40
PNYQEEWVTHKKEIR	HLA-HLA-DRB1*03:09	1DLH.pdb	-38.45	-37.44	15.42	9.20	-0.32
IILYYYELYPTMTVV	HLA-HLA-DRB1*04:01	1DLH.pdb	-65.79	-42.89	61.36	-20.32	0.00
KARNPTVTYGKNQVI	HLA-HLA-DRB1*04:02	1DLH.pdb	-32.53	-38.37	64.53	-5.14	-3.19
NQVIMLLYPDHPTLL	HLA-HLA-DRB1*04:04	1DLH.pdb	-51.03	-33.77	38.54	-10.26	-0.40
IILYYYELYPTMTVV	HLA-HLA-DRB1*04:05	1DLH.pdb	-65.79	-42.89	61.36	-20.32	0.00
EIILYYYELYPTMTV	HLA-HLA-DRB1*04:08	1DLH.pdb	-48.93	-30.22	31.99	-14.54	-1.20
YPDHPTLLSYRNMGE	HLA-HLA-DRB1*04:10	1DLH.pdb	-18.94	-31.49	37.50	-9.47	0.00
VTHKKEIRLTVPTEG	HLA-HLA-DRB1*04:21	1DLH.pdb	-28.02	-33.56	27.57	3.88	-3.35
LYPTMTVVVVSVASF	HLA-HLA-DRB1*04:23	1DLH.pdb	-65.37	-45.18	66.30	-17.71	-2.77
QVIMLLYPDHPTLLS	HLA-HLA-DRB1*04:26	1DLH.pdb	-51.13	-32.35	9.55	0.07	-0.58
TMTVVVVSVASFILL	HLA-HLA-DRB1*07:01	1DLH.pdb	-70.05	-24.65	14.82	-15.32	-0.38
PTMTVVVVSVASFIL	HLA-HLA-DRB1*07:03	1DLH.pdb	-85.20	-26.31	17.95	-19.39	-0.86
EIILYYYELYPTMTV	HLA-HLA-DRB1*08:01	1DLH.pdb	-48.93	-30.22	31.99	-14.54	-1.20
LYPTMTVVVVSVASF	HLA-HLA-DRB1*08:02	1DLH.pdb	-65.37	-45.18	66.30	-17.71	-2.77
LYPTMTVVVVSVASF	HLA-HLA-DRB1*08:04	1DLH.pdb	-65.37	-45.18	66.30	-17.71	-2.77
LYPTMTVVVVSVASF	HLA-HLA-DRB1*08:06	1DLH.pdb	-65.37	-45.18	66.30	-17.71	-2.77
EIILYYYELYPTMTV	HLA-HLA-DRB1*08:13	1DLH.pdb	-48.93	-30.22	31.99	-14.54	-1.20
EIILYYYELYPTMTV	HLA-HLA-DRB1*08:17	1DLH.pdb	-48.93	-30.22	31.99	-14.54	-1.20
LYYYELYPTMTVVVV	HLA-HLA-DRB1*09:01	1DLH.pdb	-84.98	-36.04	31.40	-16.14	-2.53
WGNNEPYKYWPQLST	HLA-HLA-DRB1*11:01	1DLH.pdb	-48.71	-36.22	13.91	-1.67	-1.91
PNYQEEWVTHKKEIR	HLA-HLA-DRB1*11:02	1DLH.pdb	-38.45	-37.44	15.42	9.20	-0.32
LYPTMTVVVVSVASF	HLA-HLA-DRB1*11:04	1DLH.pdb	-65.37	-45.18	66.30	-17.71	-2.77
LYPTMTVVVVSVASF	HLA-HLA-DRB1*11:06	1DLH.pdb	-65.37	-45.18	66.30	-17.71	-2.77
VSVASFILLSMVGVA	HLA-HLA-DRB1*11:07	1DLH.pdb	-60.96	-26.34	8.82	-7.85	-1.42
PNYQEEWVTHKKEIR	HLA-HLA-DRB1*11:14	1DLH.pdb	-38.45	-37.44	15.42	9.20	-0.32
PNYQEEWVTHKKEIR	HLA-HLA-DRB1*11:20	1DLH.pdb	-38.45	-37.44	15.42	9.20	-0.32
PNYQEEWVTHKKEIR	HLA-HLA-DRB1*11:21	1DLH.pdb	-38.45	-37.44	15.42	9.20	-0.32
LYPTMTVVVVSVASF	HLA-HLA-DRB1*11:28	1DLH.pdb	-65.37	-45.18	66.30	-17.71	-2.77
LYYYELYPTMTVVVV	HLA-HLA-DRB1*12:01	1DLH.pdb	-84.98	-36.04	31.40	-16.14	-2.53
LYPTMTVVVVSVASF	HLA-HLA-DRB1*13:01	1DLH.pdb	-65.37	-45.18	66.30	-17.71	-2.77
TMTVVVVSVASFILL	HLA-HLA-DRB1*13:02	1DLH.pdb	-70.05	-24.65	14.82	-15.32	-0.38
LYPTMTVVVVSVASF	HLA-HLA-DRB1*13:04	1DLH.pdb	-65.37	-45.18	66.30	-17.71	-2.77
LYPTMTVVVVSVASF	HLA-HLA-DRB1*13:05	1DLH.pdb	-65.37	-45.18	66.30	-17.71	-2.77
LYPTMTVVVVSVASF	HLA-HLA-DRB1*13:07	1DLH.pdb	-65.37	-45.18	66.30	-17.71	-2.77
LYPTMTVVVVSVASF	HLA-HLA-DRB1*13:11	1DLH.pdb	-65.37	-45.18	66.30	-17.71	-2.77
LYPTMTVVVVSVASF	HLA-HLA-DRB1*13:21	1DLH.pdb	-65.37	-45.18	66.30	-17.71	-2.77
PNYQEEWVTHKKEIR	HLA-HLA-DRB1*13:22	1DLH.pdb	-38.45	-37.44	15.42	9.20	-0.32
PNYQEEWVTHKKEIR	HLA-HLA-DRB1*13:23	1DLH.pdb	-38.45	-37.44	15.42	9.20	-0.32
LYPTMTVVVVSVASF	HLA-HLA-DRB1*13:27	1DLH.pdb	-65.37	-45.18	66.30	-17.71	-2.77
LYPTMTVVVVSVASF	HLA-HLA-DRB1*13:28	1DLH.pdb	-65.37	-45.18	66.30	-17.71	-2.77
TVVVVSVASFILLSM	HLA-HLA-DRB1*15:01	1DLH.pdb	79.90	-41.02	53.09	-16.97	-4.04
EIILYYYELYPTMTV	HLA-HLA-DRB1*15:02	1DLH.pdb	-48.93	-30.22	31.99	-14.54	-1.20

PTMTVVVVSVASFIL	HLA-HLA-DRB1*15:06	1DLH.pdb	-85.20	-26.31	17.95	-19.39	-0.86
NQVIMLLYPDHPTLL	HLA-HLA-DRB3*01:01	1DLH.pdb	-51.03	-33.77	38.54	-10.26	-0.40
KNQVIMLLYPDHPTL	HLA-HLA-DRB4*01:01	1DLH.pdb	-52.05	-33.74	5.64	-6.06	-0.61
TVVVVSVASFILLSM	HLA-HLA-DRB5*01:01	1DLH.pdb	-79.90	-41.02	53.09	-16.97	-4.04
PTMTVVVVSVASFIL	HLA-HLA-DRB5*01:05	1DLH.pdb	-85.20	-26.31	17.95	-19.39	-0.86

Docking studies of CHIKV MHC I epitopes with human receptor 2X40,pdb showed that the epitopes 'SVASFILLS' and 'YPTMTVVVV' are the most effective epitopes among the predicted MHC I epitopes as they are having highest binding affinities towards the human receptor 2X40.pdb. Similarly, we identified the the most effective of MHC II epitopes through the docking studies with MHC II receptors and human receptor 1DLH.pdb. Here the most effective epitopes identified were 'PTMTVVVVSVASFIL' and 'LYYYELYPTMTVVVV'. The docked complex of most effective CHIKV E2 MHC I epitopes and human receptor 2X40 were displayed in Figure 4a &4b. The docked structures of MHC II epitopes with the human receptor 1DLH are shown in Figure 5a& 5b.

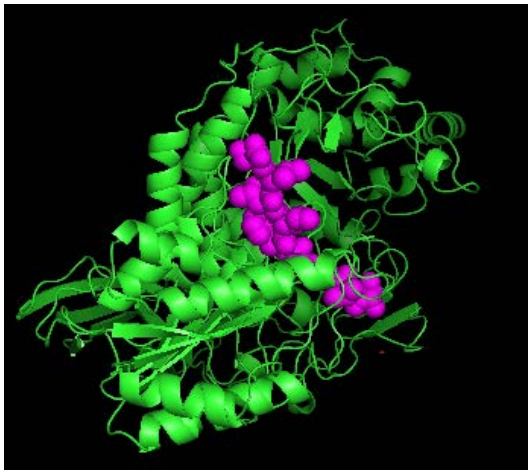

Figure 4a

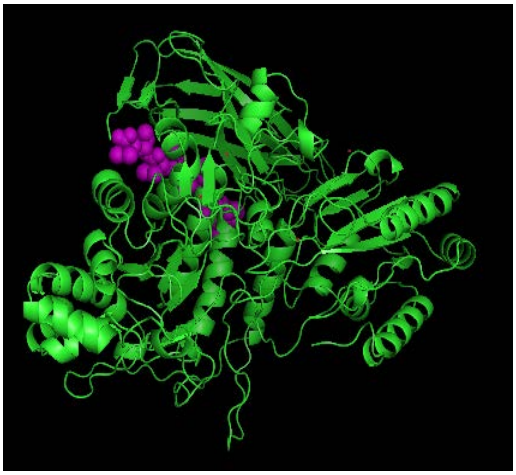

Figure 4b

Figure 4: The docked complex of most effective CHIKV E2 MHC I epitopes: 4a) 'SVASFILLS' and human receptor 2X40, 4b) 'YPTMTVVVV' and human receptor 2X40

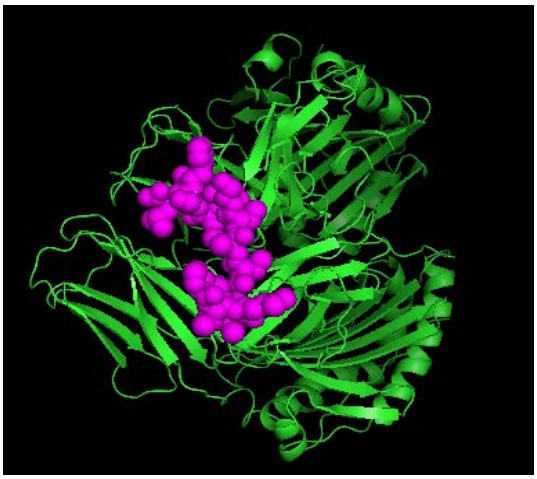

Figure 5a

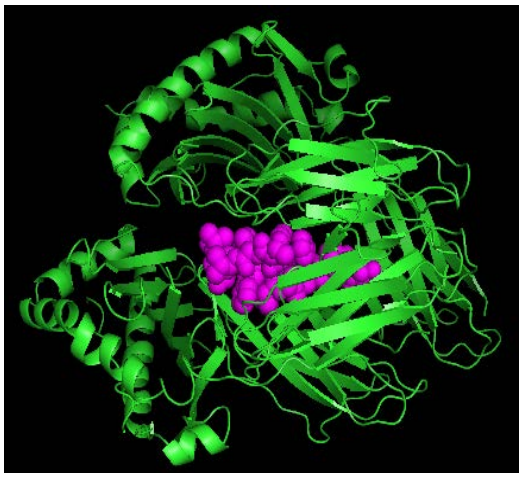

Figure 5b

Figure 5: The docked complex of most effective CHIKV E2 MHC II epitopes: 5a) 'PTMTVVVVSVASFIL' and human receptor 2X40, 5b) 'LYYYELYPTMTVVVV 'and human receptor 1DLH

IV. CONCLUSION

In this study, E2 envelope glycoprotein protein (Q0JRL8) of Chikungunya virus was analyzed for its antigenic factors. B cell and T cell Epitopes of the protein were identified and mapped using various soft computing tools. Docking studies were also conducted to analyze the binding affinities of the predicted epitopes and the most effective epitopes were identified. This work provides a better understanding of the epitopes in CHIKV E2 protein. The peptides identified as the most effective epitopes from this study can be considered for developing epitope based vaccines against Chikungunya disease.

V. FUTURE DIRECTIONS

Tiil now, there is no licensed vaccines or antiviral available for treating Chikungunya infection. The only available treatment is symptomatic, with administration of non-steroidal anti-inflammatory drugs. Recently many studies have been carried out to develop specific drugs for the infection. It was observed from a recent study conducted at NIAID that a virus-like particle (VLP) vaccine elicited immune responses in all twenty-five volunteers who participated in an early-stage clinical trial [29]. However, these attempts are its preliminary stages. The quest for the effective medicines demand further researches in this area. This study identifies some antigenic epitopes of E2 glyco protein of CHIKV. In vivo studies are required to study the elicited immune responses of these identified antigenic peptides and also to test the efficacy of these antigenic peptides as vaccine candidates. Moreover, the in vivo pathogenesis of Chikungunya virus is only poorly understood, so future research in this area is sorely needed to elucidate the *in vivo* pathogenicity of Chikungunya virus.

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