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In silico Epitopes design against Chikungunya Virus

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Abstract:

Chikungunya virus has been one of the most serious concerns in recent decades due to its rapid global expansion. Symptoms include a high fever, maculopapular rashes, intense joint and muscular pain, severe headaches, nausea, and fatigue. As it is a viral infection, a vaccination could be considered a promising treatment. In this study, peptide epitopes were created to target the structural polyprotein of CHIKV virus. The various domains of the structural polyprotein were examined for B-cell and T-cell epitopes. A total of 43 B-cell epitopes were identified, among which 'MCMCARR' had the highest score and antigenicity. T-cell epitopes, including MHC class I 'VQDISATAMSWVQK' and MHC class II 'VVLCVSFSR', are highly antigenic, non-allergenic, conserved, and can bind with multiple HLA alleles. In total, ten T-cell epitopes (5 MHC-I and 5 MHC-II) were chosen for three-dimensional structural modelling and molecular docking with HLA alleles and the TLR 3 receptor to assess their physicochemical characteristics. The HDOCK server was utilized for molecular docking, and the Chimaera and Pymol molecular graphics systems were used to visualize three-dimensional models and the docking process of the predicted epitopes with TLR 3 and T-cell epitopes in the HLA binding pocket.

Key words: Chikungunya virus, Epitope mapping, Immunoinformatics, Antigenicity, Molecular docking

Introduction

Chikungunya (CHIKV) virus has been a big issue for decades as it spreads like wildfire all over the world [1]. The term Chikungunya is derived from the Makonde word "kungunyala," which means "that which bends up" [2], describing the bent posture and trouble walking that patients have because of severe joint pain. [3]. CHIKV is an etiological agent that belongs to the Alphavirus genus of the Togaviridae family [4], which is similar to an arthropod-borne virus [5]. The CHIKV virus, which causes symptoms similar to dengue fever, has caused massive outbreaks in Africa, Asia, and Central and South America. CHIKV infection causes a rapid rise in temperature (39°C to 40°C), maculopapular rashes, severe joint pain [6; 7], muscular pain, headache, nausea, and fatigue [8]. Infection with CHIKV triggers a series of systemic innate responses, including the production of antiviral IFN and a slew of proinflammatory cytokines, chemokines, and growth factors [9; 10].

The CHIKV virus genome is a positive-sense single-stranded RNA with 11,805 nucleotides, divided into two open reading frames (ORFs) separated by a 65-nucleotide untranslated region [12]. The first open reading frame (ORF) encodes four non-structural proteins (nsP1, nsP2, nsP3, and nsP4) involved in RNA synthesis and processing [13]. The second ORF encodes structural polyproteins, including Capsid, E1, E2, E3, and 6K, which are then translated into mature proteins necessary for viral assembly [14, 7]. These structural proteins combine with the genomic RNA to form a nucleocapsid, while the E1 and E2 glycoproteins are crucial for the virus to enter cells and interact with host receptors [17, 15]. The E1 protein wraps around E2 to help form trimeric spikes on the virus's surface [18].

Developing a CHIKV vaccine is crucial for several reasons. Recent studies highlight CHIKV as a major global health issue, causing significant morbidity and economic impact in South Asia, Southeast Asia, South America and Africa [3, 26]. The virus leads to severe, chronic arthralgia, making effective vaccination essential for controlling its spread [35]. Peptide-based vaccines can offer targeted, long-lasting protection. A well-designed vaccine could greatly reduce CHIKV prevalence and severity, lessening its public health and economic impact [17, 28].

Our aim is to harness advanced in-silico epitope prediction and bioinformatics tools to develop a broad-spectrum vaccine for Chikungunya virus (CHIKV), addressing the urgent need for effective preventive measures against this debilitating viral infection. By utilizing sophisticated sequence analysis, predictive algorithms, and structural modeling [31], we aim to identify and validate immunogenic epitopes that can elicit robust immune responses across various CHIKV strains. Integrating these bioinformatics approaches allows us to optimize epitope selection for enhanced immunogenicity and cross-protection [34], ultimately contributing to a comprehensive vaccine that can safeguard global health against CHIKV.

Method

The primary sequence of Chikungunya structural polyprotein was retrieved from NCBI [19]. There chemical and physical properties were assessed using an online tool "Protparm" [20], while "Prosec" was used for prediction of helices, loops, and strands [21]. The TMHMM tool was used to assess transmembrane topology, while the targeted protein secondary structure was predicted using "Pfam" software [22]. The Vaxijen 0.2 server was used to determine antigenicity [23]. The three-dimensional structure of the CHIKV structural polyprotein was predicted using a homology modelling approach in MODELLER v.9.12 (https://swissmodel.expasy.org) [4].

B-cell Epitope Prediction

B-cell epitopes were predicted using various tools from the immune epitope database analysis resource (IEDBAR) (http://tools.iedb.org/bcell/), taking into account antigenicity, surface accessibility, and hydrophobicity [24]. Initially, linear epitopes were predicted using Bepipred 0.2, with the threshold set to 0.5, based on protein sequence's average value of 0.482. The Emini Surface Accessibility Prediction Tool was used to predict B-cell epitopes at a 1.00 threshold [25]. The Kolaskar and Tongaonkar antigenicity prediction tool was used to identify epitopes based on the antigenicity of predicted peptides [26]. Using the Ellipro server from the IEDB Database (http://tools.iedb.org/ellipro), we predicted that discontinuous B-cell epitopes will improve the variety and specificity of B-cell epitopes [27].

T-cell Epitopes Prediction

The MHC class I binding peptide was retrieved using the MHC-I binding prediction tool from IEDB (http://tools.iedb.org/mhci/) [28]. This server uses artificial neural networks algorithms to compute epitopes based on binding affinity. All human alleles for the entire length were selected and reduced to nine amino acids. MHC-II peptides were assessed by the MHC-II binding prediction tool from IEDB (http://tools.iedb.org/mhcii/) and the NtMHCPan 4.0 prediction method [29]. Protein sequences were submitted in Fasta format, and a reference set of seven HLA-DR alleles and several sets of HLA-DQ alleles, which have binding affinity with the CHIKV polyprotein, were selected for full length.

Antigenicity, Allergenicity, and Toxicity Assessment

Predicted epitopes were screened for antigenicity using VaxiJen 0.2 server (http://ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) [30]. AllergenFP server (https://ddgpharmfac.net/AllergenFP/) was used to select the best non-allergic candidate vaccination. All predicted MHC-I, MHC-II, and B-cell epitopes with default parameters were submitted to AllergenFP, and only non-allergic peptides were selected for further study [31]. ToxinPred (http://crdd.osdd.net/raghava/toxinpred/) calculated the toxicity of all predicted epitopes, while only non-toxic epitopes were selected for further study [32].

T-cell Epitopes Three-Dimensional Structure Prediction and Molecular Docking

After analysis, all the predicted non-allergic, non-toxic, and antigenic T-cell epitopes were submitted to the PEPFOLD server (http://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD/) for three dimensional structure prediction [33]. The HDOCK server(http://hdock.phys.hust.edu.cn/) and HPEPDOCK web servers were used to perform molecular docking between selected epitope models with their associated HLA alleles and TLR-3 receptors. Three-dimensional models of HLA-A/B, HLA DR, HLA DB, and TLR-3 were downloaded from the RCSB Protein Data Bank (PDB) (https://www.rcsb.org/) and docked against their respective epitopes [34].

Results

Primary Structure Analysis

The primary sequence of Chikungunya structural polyprotein with Accession number **(QJW82716)** was retrieved from NCBI (http://www.ncbi.nlm.nih.gov/) in FASTA format**,** recently reported containing 1248 amino acids. To establish the sequence physical and chemical properties, we used the ProtParam online server. The results showed that the molecular weight of the sample protein was 138,358.96 kDa, with an isoelectric point of 8.88. Positively charged proteins have an isoelectric point greater than 7. The analysis revealed 107 negatively charged amino acids (Asp + Glu) and 138 positively charged amino acids (Arg + Lys). The instability index was computed to be 41.46, indicating that protein as stable [37], while being close to the threshold. The aliphatic index, which measures the protein's thermostability, was 74.78, indicating moderate stability under various conditions. Additionally, the Grand Average of Hydropathicity (GRAVY) was found to be 0.308, suggesting that the protein is slightly hydrophobic, which may affect its solubility and interaction with water. The 3D structure of the CHIKV polyprotein was modeled using the Swiss-Model server (https://swissmodel.expasy.org/) and visualized with UCSF Chimera and PyMOL (Figure 1). I-TASSER server characteristics, such as C-score (−1.03), TM score (0.59 ± 0.13), RMSD (13.2 ± 4.3 Å), and cluster density (0.1358), were assessed to ensure structural accuracy. The PROCHECK service was used to assess the stereochemical quality of the predicted model; the Ramachandran plot statistical findings show that 87.3% are in the most favored regions, followed by 12.9% additional allowed region, 3.5% generously allowed reand 2.3% disallowed region.

B-cell Epitopes

Linear B-cell epitopes were predicted with BepiPred 2.0 (http://tools.iedb.org/bcell/). The BepiPred tool is trained with a Random Forest algorithm that predicts B-cell epitopes from protein sequences containing both epitopes and non-epitope amino acids. Residues with scores above the threshold (0.5) were considered as to be part of B-cell epitope.

BepiPred predicted a total of 34 potential epitopes, which were further screened on the VaxiJen server for selection of antigenic epitopes, totally (20) B-cell epitopes were selected, which have a higher probability of invoking B-cell immune response. The results are summarized in Table 1. Discontinuous B-cell Epitopes were predicted using ElliPro (http://tools.iedb.org/ellipro/) tools from the IEDB Server. The three-dimensional structure Model of the structural polyprotein was submitted and all four chains were selected (Figure 2). The resulting predicted discontinuous B-cell epitopes were identified and mapped on the three dimensional structure of the vaccine (Figure 3).

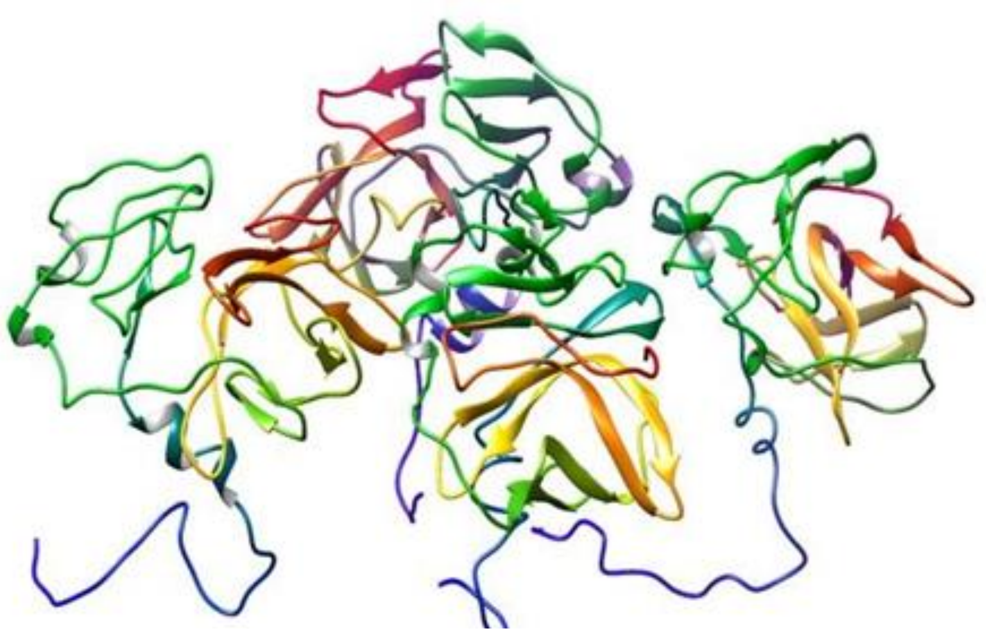

Figure 1. Three-dimensional structure of CHIKV structural polyprotein visualized with Chimera

No.	Start position	End position	Predicted B-cell Epitope	Peptide length	Vaxijen score
1	203	220	IPTGAGKPGDSG	12	0.52
2	252	269	KITPEGAEE	9	0.91
3	319	330	SPHRQRRSTKDN	12	0.54
4	361	368	RIRNEATD	8	1.05
5	382	389	KTDDSHDW	8	0.67
6	453	462	PFHHDPPVIG	10	0.84
7	559	577	KWQYNSPLVPRNA	13	1.38
8	726	732	ELTPGATAR	9	1.68
9	875	884	AECKDKNLPD	10	1.25
10	876	885	ECKDKNLPDY	10	1.07
11	993	1023	GQFGDIQSRTPESEDVY	31	1.01
12	976	983	SSAWTPFD	8	0.78
13	992	1025	DVYNMDYPPFGAGR	34	0.93
14	1101	1107	DAPSLTD	7	0.60

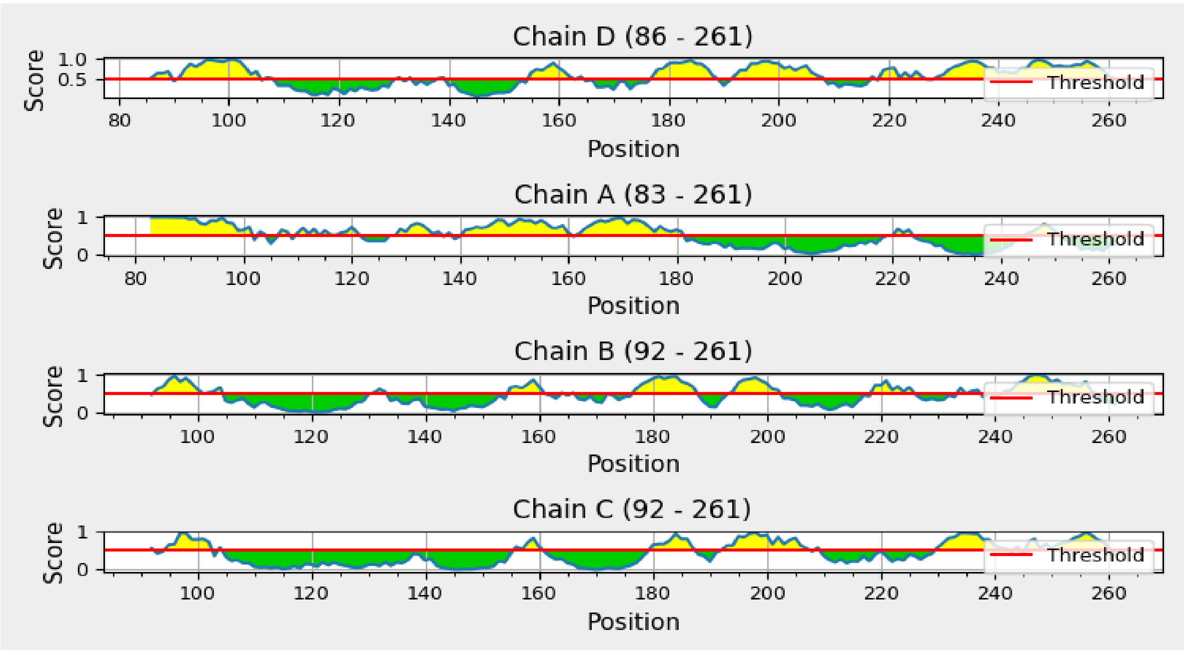

Figure 2. Protrusion Index Chart illustrating protruded residues (predicted discontinuous B-cell epitopes) from all A, B, C, and D chains of the subject protein. The red line indicates the threshold, while the yellow peaks highlight the location and presence of epitopes.

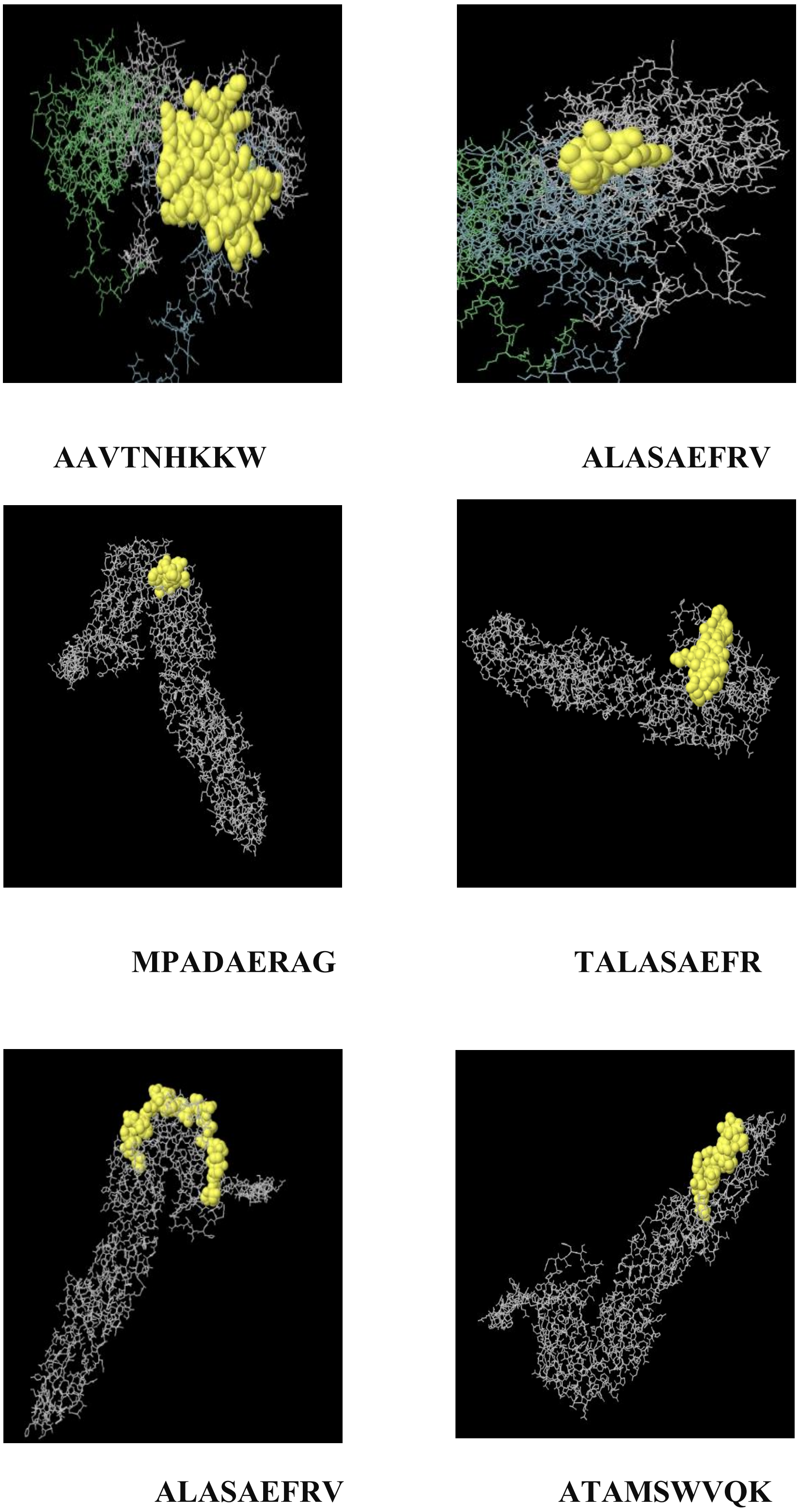

Figure 3. Three-dimensional structure representation of discontinuous epitopes of CHIKV structural polyprotein. The epitopes are highlighted by yellow colored surface, and the grey sticks represents bulk polyprotein.

T-cell Epitopes Prediction

The MHC-I epitopes were predicted using the MHC-I binding prediction tool from IEDB (http://tools.iedb.org/mhci/) and the Proteasomal cleavage, TAP transport, MHC class I combined predictor. NetMHCpan 4.0 prediction method was selected. The sequence was provided in FASTA format, with 19 HLA-A/B alleles. Epitopes with lower IC50 values have a higher affinity for binding to the MHC molecule. MHC Class-I epitopes with high proteasomal scores (1.8-1.1), TAP scores (1.4-1.29), and IC50 values of less than 50 nM (IC50 < 50 nM) were selected. All the selected epitopes were submitted to the IEDB-AR v.2.22 MHC-I immunogenicity tool (http://tools.iedb.org/immunogenicity/) for immunogenicity analysis, and 29 immunogenic epitopes were selected for further analysis.

All predicted epitopes were submitted to Vaxijen and ToxinPred with default parameters, only 11 peptides were toxic while 18 peptides were non-toxic, these NT peptides were selected for further study. AllergenFP was used for selection of non-allergic epitopes. The epitopes with the highest total score are not the best epitopes. We selected epitopes that had high binding affinity, highly antigenic, 100% conserved, non-allergenic, nontoxic can bind to several alleles (Table 2).

MHC class-II epitopes were predicted using the MHC-II binding prediction tool from IEDB. NtMHCPan 4.0 prediction method was selected , For HLA- DR binding peptides prediction, a 7-allele human leukocyte antigen (HLA) reference set was selected and for HLA-DQ four sets of separate alpha and beta chains (HLADQA1 *05:01/DQB1*03:01,HLADQA1*05:01/DQB1 *02:01,HLA,DQA1*03:01/DQB1*03:02,HLA-DQA1*01:01/DQB1* 05:01) were selected from reference HLA alleles. Onıy 59 antigenic epitopes above the threshold were selected, and "YELTPGATV" peptide was found highly antigenic with 1.39 VaxiJen score. Further 19 non-allergic and non-toxic epitopes were selected for vaccine design (Table 3). Three-dimensional structure of T-cell epitopes was predicted via PEP-FOLD, best models of Epitopes were filtered on the base of scoring criteria for molecular docking (Figure 4-5).

Population Coverage

For the prediction of population coverage five MHC I and five MHC II epitopes and their respective HLA alleles were considered. MHC I presented 91.23% and MHC II presented 83.4% of the global population. In West Africa, the vaccine demonstrated a predicted coverage of 87.95% for MHC Class I and 81.41% for MHC Class II. Conversely, in South Asia, the predicted coverage was 83.47% for MHC Class I and 78.65% for MHC Class II. Peak coverage of MHC I Epitopes observed in the people of Europe (96.25%), North America (92.23%) and North Africa (90.17%). Whereas peak coverage of MHC II epitopes was observed in North America (93.96%) and South America (92.94%) (Figure 6).

Table 2. Antigenicity, Allergenicity, Toxicity, and Conservancy analysis of Predicted T-cell MHC Class-I Peptides (epitopes).

Peptide	Antigenicity	Immunogenicity	Toxicity	Allergenicity	Conservancy
ALASAEFRV	0.994	0.24774	NT	NA	100%
ALSVVTWNK	0.697	0.23391	NT	NA	100%
ATAMSWVQ	1.0529	0.19052	NT	NA	100%
LTCSPHRQR	0.735	0.10762	NT	NA	100%
SPLVPRNAE	1.0628	0.09326	NT	NA	100%
TALASAEFR	1.1988	0.0644	NT	NA	100%
VVLCVSFSR	1.568	0.04941	NT	NA	100%
AAVTNHKK	0.9838	0.0185	NT	NA	100%

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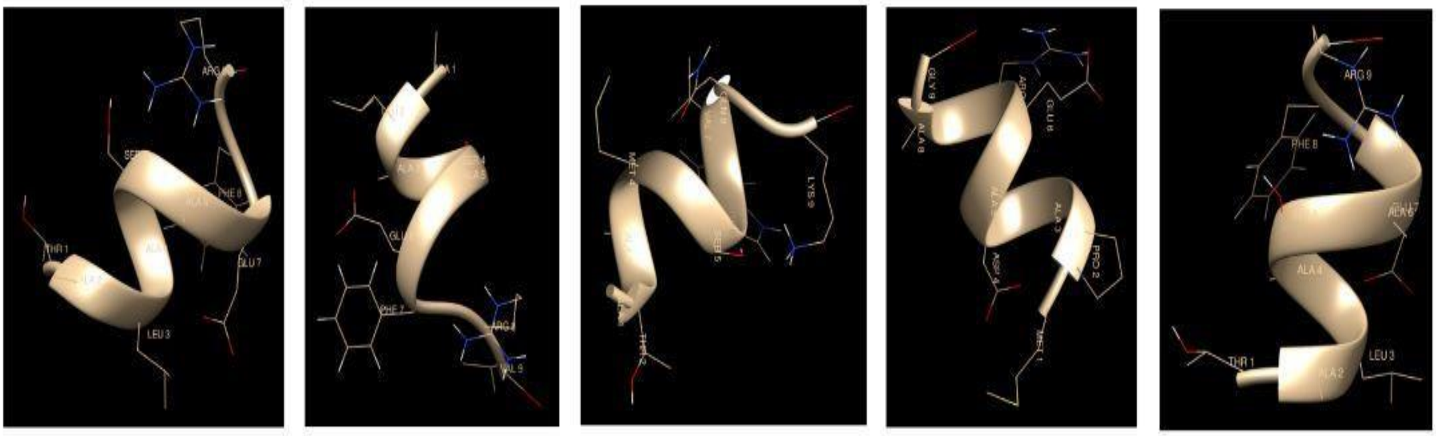
VVLCVSFSR	1.568	0.04941	NT	NA	100%
A[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Table 3. Predicted T-cell MHC Class-II Peptides (epitopes) binding to HLA DRB alleles with IC50 Score.

Peptide	Vaxijen Score	Toxicity	Allergenicity	Conservancy
IILYYYELYPT	0.8151	NT	NA	100%
ISFSTALASAE	0.7368	NT	NA	100%
PPFGAGRPGQF	1.0479	NT	NA	100%
PTGAGKPGDSG	1.31	NT	NA	100%
QKITGGVGLVV	0.8951	NT	NA	100%
VTIREAEIEVE	1.3547	NT	NA	100%
WVQKITGGVGLVVAV	0.74	NT	NA	100%
LQISFSTAL	0.85	NT	NA	100%
RRCITPYEL	0.76	NT	NA	100%
YELTPGATV	1.39	NT	NA	100%



AAVTNHKKW ALASAEFRV ATAMSWVQK MPADAERAG TALASAEFR

Figure 4. Three-dimensional structure of predicted MHC class-I Epitopes with PEP-FOLD server.

LQISFSTAL VEGNSQLQI YRAHTASASAK WVQKITGGVGLVVAV QKITGGVGLVV

Figure 5. Three-dimensional structure of predicted MHC class-II Epitopes with PEP-FOLD server

Docking Analysis

Five MHC Class-I and five MHC Class-II peptide models were chosen and submitted for molecular docking with their respective HLA alleles. Molecular structures of HLA alleles were acquired from RCSB in PDB format and these three-dimensional structures were further analyzed using molecular visualization software, such as UCSF Chimera and PyMOL. Epitopes docking was performed using the HDOCK server. The analysis was performed manually. The best binding pockets were selected based on the docking and LG scores. T-cell-receptors (TLRs) attach to antigenic molecules presented by major histocompatibility complex proteins and play an important role in cellular immune responses. Molecular docking between predicted T-cell epitopes and Tool-like receptor TLR3 was performed using Haddock server. The structural model of TLR 3 with PDB ID 1ZIW was downloaded from PDB and submitted to HDOCK server with predicted T-cell epitope. To determine TLR3 complex's binding affinity, a Gibbs free energy analysis was performed. The compound showed a ΔG value of -19.5 kcal/mol. A negative ΔG value indicates a thermodynamically feasible complex. This suggests that the epitope-TLR-3 interaction is energetically favorable, persistent, and likely to occur spontaneously as shown in the (Figure 8).

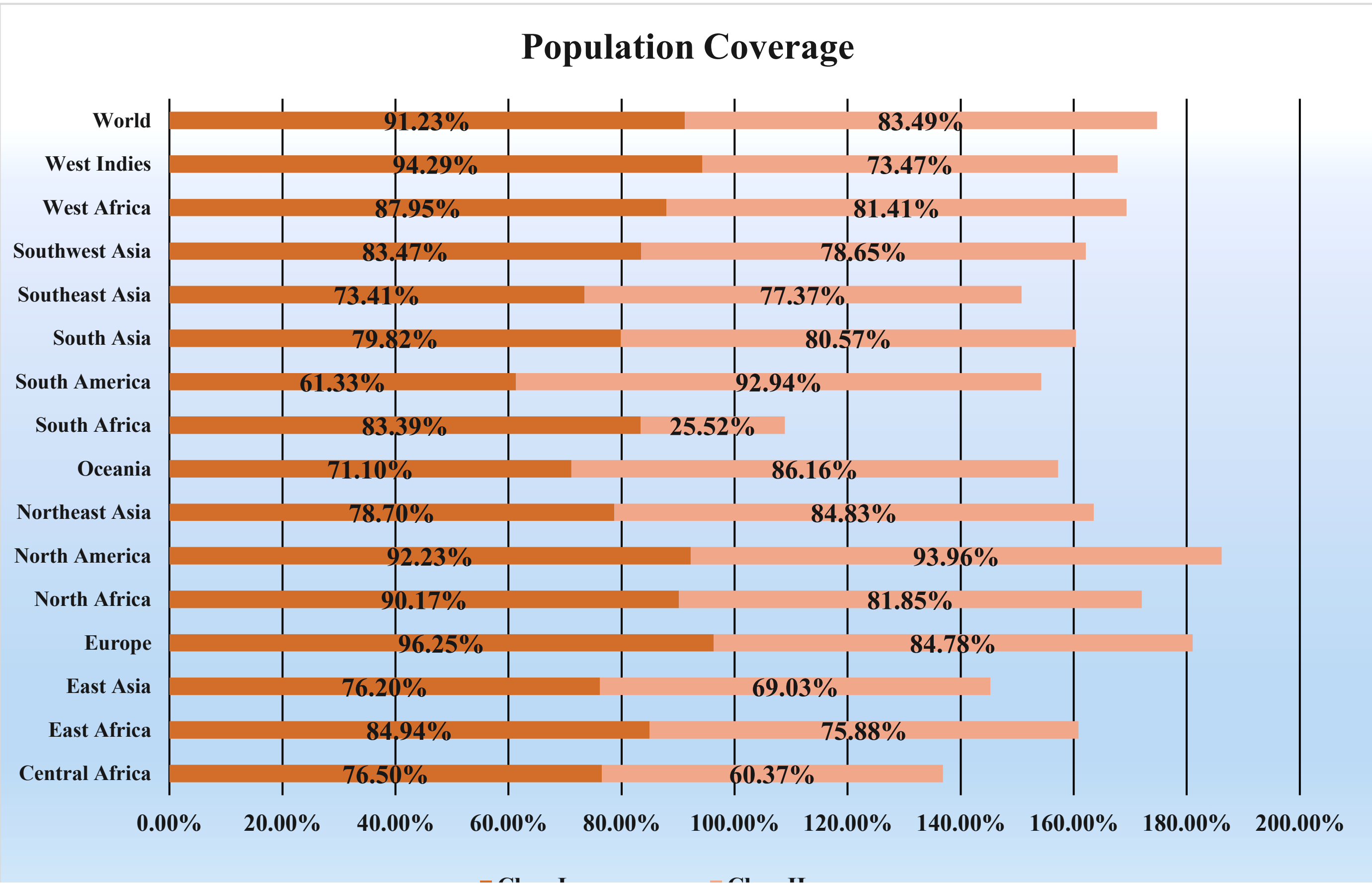


Figure 6. Population coverage of both MHC-1 and MHC-II epitopes in different regions of world.

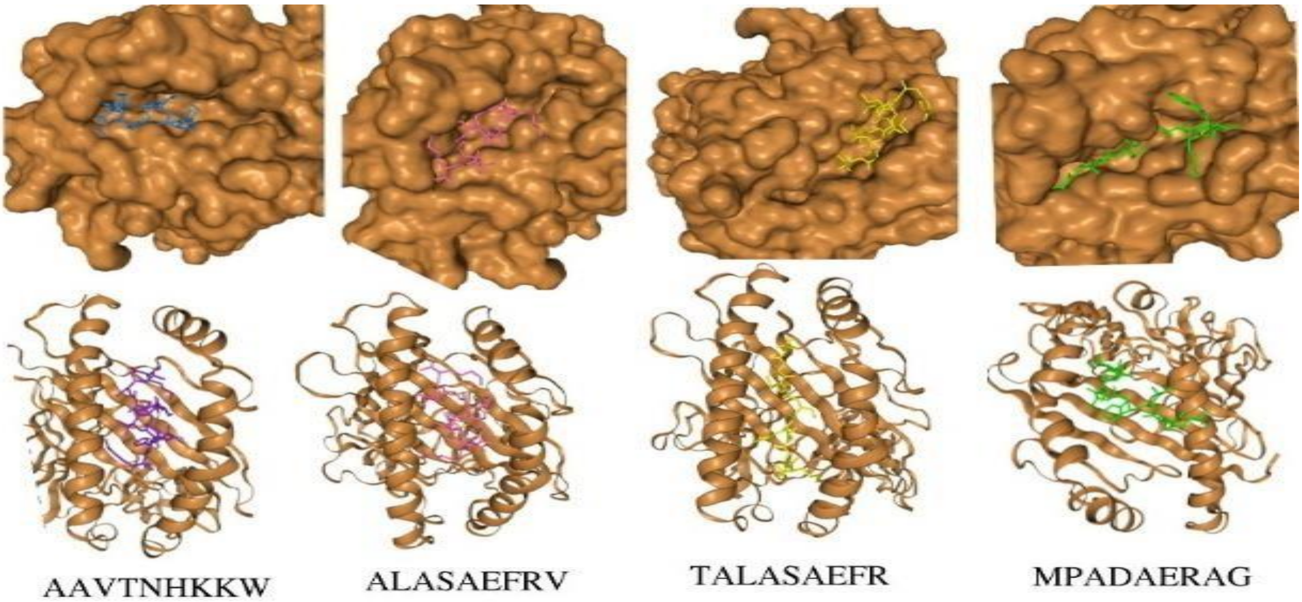


Figure 7. The three-dimensional binding pattern of the selected 5 MHC class I peptides (colored) docked with their respective HLA alleles (brown) at binding site.

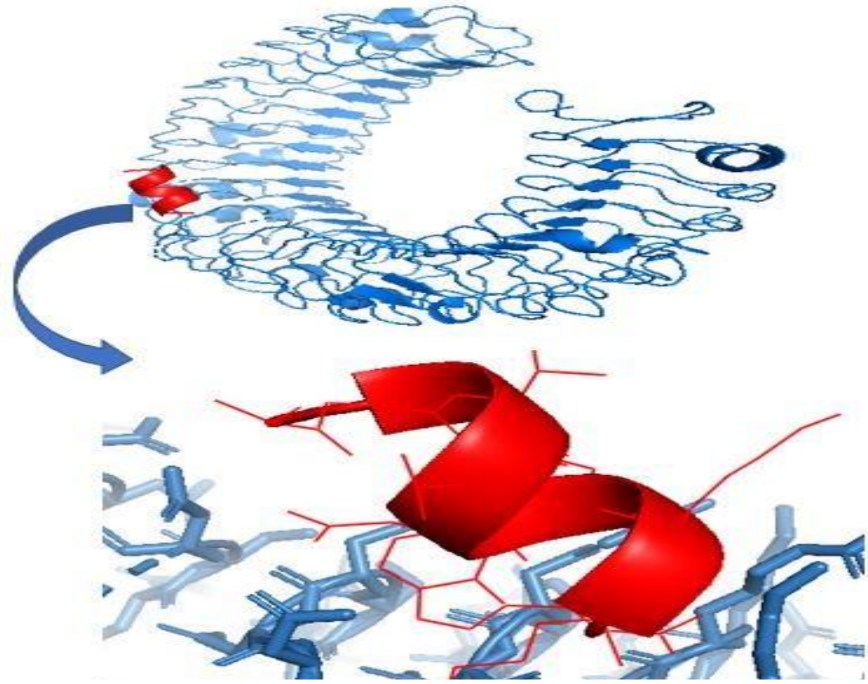


Figure 8. Molecular interaction pose of docking complex of the epitope-TLR3 refined cluster. The designed epitope is colored red while the TLR3 structure is colored light blue. Residues that interact are concentrated.

Conclusion

Chikungunya is a serious viral infection with no current cure, prompting scientists to explore various biological approaches for its prevention and treatment. One promising strategy involves the development of vaccines, particularly multiple epitope vaccines, which could serve as effective treatment options. In this study, we utilized immunoinformatics approaches to predict potential antigenic epitopes on the CHIKV virus's structural polyprotein, a critical component for viral attachment and entry into host cells.

Our analysis included the examination of the primary, secondary, and tertiary structures of the target protein, revealing that it is thermally stable and hydrophilic. We identified thirty-seven linear B-cell epitopes, with 'ELGDRKG' having the highest antigenicity score. Additionally, conformational B-cell epitopes such as 'DLECAQIPVH' and 'ELTPGATAR' emerged as strong candidates. For T-cell epitopes, MHC Class I and II binding predictions highlighted "VVLCVSFSR" and "ALASAEFRV" for Class I, and "VTIREAEIEVE" and "QKITGGVGLVV" for Class II as having significant binding affinities. Molecular docking results confirmed strong interactions between these predicted epitopes, HLA alleles, and the TCR receptor protein.

All candidate epitopes were rigorously assessed for their antigenicity, allergenicity, toxicity, conservancy, and population coverage, leading to the selection of the best candidates for a broad-spectrum vaccine. The designed CHIKV vaccine demonstrates considerable potential in addressing multiple strains of the virus and providing robust, long-lasting protection. By targeting conserved and immunogenic epitopes, this vaccine offers a promising solution that could surpass current treatments. This study paves the way for the future development of novel peptide vaccines to combat CHIKV infection globally, potentially reducing the public health impact of this re-emerging disease.

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