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USING IMMUNOINFORMATIC APPROACH DESIGN EPITOPE-BASED VACCINE FOR CHIKUNGUNYA VIRUS

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ABSTRACT: Chikungunya is a mosquito-borne infection of the genus alphavirus. It is vaccines, medications, and other treatments difficult to produce. The CHIK-Virus is carried thru the mosquito, which functions as a vector for the disease. Vaccines, therefore, provide alternate preventative techniques for the spread of viral infection. Structural proteins of CHIK-Virus with T and B cells had been used for immuno-informatic approach. The identification of epitopes is a critical step in the development of vaccines against infectious illnesses. The author of this research work used scientific forte databases to choose the binding interactions of the epitopes PLVPRNAEL and RNEATDGTL have been created for a vaccine that contained non-toxic, non-allergenic, however tremendously antigenic determined in Asia, Africa, and Central South America. As a result of it's far a high-quality feel polymer virus. The replication of CHIKV in the host results in genome mutation, making T-cells from CHIKV. Finally, epitopes acknowledged thru molecular docking research can be wont to supply huge spectrum vaccines, which might be powerful in opposition to numerous lines of Chikungunya.

INTRODUCTION: Dengue, CHIK, Zika, Nipah, and COVID-19 are just a few of the vector-borne infectious illnesses that have recently become a major public health problem [1, 2, 3, 4]. *Aedes aegypti* and Albopictus, two mosquitoes that transmit the Chikungunya virus to people, belong to the Togaviridae family. Major Chikungunya outbreaks in the South Republic of India in 2007 and 2008, CHIK virus with new genetic changes had been detected. Chikungunya fever has been in massive global jeopardy for the reason that mid-1950s.

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Chikungunya virus (CHIKV) contamination due to an arthropod-borne virus turned into first found in Africa, CHIK-Virus additionally constrained in Southeast Asia. Epidemics in the West Pacific Islands additionally had been observed. Viral pathogenesis can be caused by cellular malfunction and or cell death. Several cell death routes have been identified, each of which may occur on its own [5].

The CHIKV contamination reasons a huge variety of scientific symptoms, such as fever, headache, nausea, tiredness, and muscular ache, in addition to paralysing joint aches, high-grade fever, conspicuous rashes and polyarthralgia that may close from days to months [6]. The inflammation caused during the acute phase of CHIKV infection may reduce erythrocyte half-life (RBCs). Clinical data reveal that anaemia is commonly diagnosed in

individuals suffering from severe chikungunya [7]. Clinical manifestations collectively with myalgia, vomiting and joint ache also are seen. After some months of acute contamination, the CHIK-Virus additionally restrained Dengue-like medical symptoms . When collagen smash then metabolism of connective tissues in cartilage, reasons extreme acute arthritis [8]. There's currently no permitted medical care or vaccine for the remedy of chikungunya contamination at the market. Antiviral activity against CHIKV has been documented in a wide range of traditional medicinal herbs and plants [9]. Efforts to be seeking for out accomplice degree trivial drug treatments are nevertheless inside the early stages. Because there is no particular cure for Chikungunya and the disease has such a large effect, experts recommend using monoclonal antibodies, nucleic acid modifiers, designer molecules and secondary phytochemicals [10]. Till long way antipyretic drug treatments to lessen physical aches and fevers [11]. No remedy or immunization is presently available [12]. So there maybe want to evolve a separate vaccine for such an infectious illness. Proving that CHIKV neutralizing antibodies against one genotype are quite efficient against the other genotypes [13].

CHIK-Virus had a single-stranded RNA; this is 11.8 kb in massive, which includes 11,990 base pairs. CHIK-Virus carries 2 open reading frames (ORF) and quick non-translatable sections. The goal of non-structural protein maximum a hit strategy for growing antiviral remedies; however, its hindrance for viral replication is a large hassle for vaccine development. In comparison to different alpha viral proteins, they are extraordinarily tough to track. In my studies, we hire the CHIKV structural polyprotein that collects right into a viral particle supplying the immune system with an appropriately folded tertiary structure [14, 15]. The purpose of a vaccine is to neutralize antibodies with evidently received shielding immunity. Antibodies like those are taken into consideration to be the most important mediators of safety [16, 17]. In addition to the genomes of different species, the human genome is being tested end result huge immunogenic records are to be had now. My purpose is to use contemporary immunogenic records and immunogenic tools to supply conserved B cell and T cell epitopes the CHIKV structural polyprotein that may expand a CHIKV vaccine [18]. This study makes use of a computational approach to observe the precise genetic traits of B cell and T cell epitopes to supply immunogenic response and decrease viral exposure [19.] The CD4 and CD8 cells help withinside the processing of the certain antigen with the aid of using that kill the antigen and affords vital safety to the host mobileular [20]. Molecular analysis is used for the interplay of epitopes and MHC alleles. A promiscuous T mobileular epitope is a project with withinside the introduction of peptide-primarily based totally vaccinations [21]. Docking evaluation turned into used to perceive some of the promising applicants to be used as a diagnostic agent for mosquito-borne ailments together with malaria and chikungunya [22].

MATERIALS AND METHODS:

2.1. Viral Protein Selection for Vaccine Preparation: The amino acid sequence of the structural polyprotein of CHIKV was acquired from the N C B I database. The proteins of the infectious pathogen were extracted using the FASTA format.

2.2. Prediction of Epitopes from Shortlisted Proteins: ProPred1 is a web-based program that predicts MHC binding in antigenic sequence data using a graphical interface.

Propred1 was utilized to locate T mobileular epitopes with an excessive affinity for sophistication HLA alleles [23]. Epitopes are shaped while antigenic fragments are coupled to MHC Class II proteins, which T-helper cells (CD4) can recognize. So MHC Class II proteins are very important.

2.3. Predictions of Instability, Aliphatic Index and GRAVY Analysis: Prot Param is a software program that calculates physical and chemical properties like stability for a protein in TrEMBLL in addition to a user-entered epitope sequence [24]. The relative quantities stuffed with the aid of using aliphatic facet chains are defined as the aliphatic index of a protein (alanine, valine, isoleucine, and leucine). One of the computed metrics is the grand average of hydropathicity (GRAVY) evaluation of the maximum common epitope during the extracellular matrix. As GRAVY values a more terrible rating chosen.

2.4. Antigenicity, Immunogenicity and Allergenicity Prediction: Antibodies react with epitopes, causing the immune system to build a defense mechanism to combat new threats. Without utilizing alignment, VaxiJen is the first server to forecast protective antigens [26]. IEDB is a user-friendly program that provides a complete database for experimentally measuring, predicting, and analyzing epitope immunogenicity [27]. The Allergen FP method, which was mentioned in this study, was included (FP stands for Finger Print). If>35 % of series identical throughout an 80-amino-acid range, in preference to recognized allergens [28].

2.5. B-cell Epitope Prediction: Structural polyproteins were examined by ABC Pred for the prediction of T cell epitopes that are part of B cell epitopes. B cell better equipped to neutralize the antigen's impact [29]. The predicted epitopes were further narrowed down based on their prediction score.

2.6. Prediction of Shot Listed Consensus Epitope: The prediction of common epitopes among known serotypes might be used to produce Chikungunya vaccines. The standard peptides were revealed to be consensus epitopes when the predicted CHIKV serotype epitopes were examined. The consensus epitopes elicit an immune response against the Chikungunya Virus. This analysis only considered T cell epitopes that were partially linear B cell epitopes for further investigation.

2.7. Prediction of Toxicity, Hydrophobicity, Hydropathicity, Hydrophilicity with Charge and Mol. Wt: Toxin Pred is getting used to assess epitope toxicity, hydrophobicity, hydropathicity, and hydrophilicity with terms of epitope charge and molecular weight [25]. It can be used to perceive the no toxic peptides for study.

2. 8. Modeling, Refining, and Validation of Tertiary Structures: PEPstr is a program that predicts the tertiary structure of small peptides with 7 to 25 residues in length. This is a service that generates three-dimensional representations of peptide structures in nanoscale.

2. 9. Structure-Based Modeling and Evaluation of MHC Alleles: The HLA allele sequence was obtained from the IMGT/HLA database [30, 31]. The sequence in which no access to the structure was available. The structure of HLA alleles was modeled using Modeler 9.10. The template for creating the model was chosen using BLAST and downloaded from the PDB database. Procheck was the software that evaluated the stereochemical efficiency of a protein structure.

2.10. Prediction of IC_{50} Conservancy and Docking Analysis: Propred 1 predicted the relevant allele hypothesized T-cell epitopes for each of the genes based on IC_{50} values. Only variable allele frequency was measured and tallied to fulfill the needs and demands of binding alleles. The redundant alleles were ignored completely. To determine epitope conservation, the IEDB's epitope conservancy research technique was used to anticipate the target epitopes conservancy trend [32]. Identification factors can be used to compute conservancy. Using the IEDB conservancy tool, all predicted Consensus T cell epitopes of chikungunya were analysed for conservancy among all accessible genotypes of chikungunya [33]. The discovered epitopes that were non-numeric in size had an IC_{50} value less than 50 and a conservancy value of 88–100% were chosen for further investigation. The HPEPDOCK server used as a hierarchical docking method for Protein–peptide docking often necessitates an extensive look for viable binding modes during the entire protein [34]. For healing capabilities require identifying protein-peptide complexes taking part in those interactions [35]. The server ensemble docking of produced peptide conformations to the Protein.

2.11. Population Coverage Analysis: The population coverage of the selected epitopes was calculated using the IEDB tool. Due to the high variability of MHC molecules, individuals of various ethnicities/countries have varied MHC-related pools/frequencies. Consequently, epitope-based vaccines might be utilized to enhance population distribution by lowering the amount of uncertainty and variance in coverage achieved or predicted among ethnic groups.

RESULTS:

3.1. Viral Protein Selection: NCBI database used (Accession No: AYW 76678.1) for this study, structural proteins from CHIKV serotypes were

chosen, and an epitope was created that elicited both B and T cell responses.

3.2. Epitope Estimation for Cytotoxic T Lymphocytes (CTL) and Helper T lymphocyte (HTL): CTL epitopes projected from the propried 1 tool were used to classify the most promiscuous epitopes relative to the MHC category I allelomorph for CHIKV superantigen. All structural protein epitopes are combined with a longer chain of peptides. The propriety server for MHC II predicted Helper T lymphocyte (HTL) epitopes for structural Protein. Total 26, 28 Cytotoxic lymphocyte T cell epitopes for CHIKV were removed for study.

3.3. Predictions of Instability, Aliphatic Index and GRAVY Analysis: Instability index discord unstable epitopes in cytotoxic and helper T lymphocytes. For the subsequent research, 20 stable epitopes had been utilized, while 6 epitopes had been discarded for T cytotoxic. In addition, 21 stable epitopes had been utilized; whearas7 epitopes had been discarded for T lymphocytes. All excellent Aliphatic index values protected inside aspect by examine will increase thermostability.

Prot Param is an outstanding protein collection database that has been meticulously maintained. We observed thirteen CTL and eight HTL epitopes with better terrible value through GRAVY analysis. This technique became utilized to pick out research with a better GRAVY terrible score and stability 8,4 cytotoxics, lymphocytes epitopes finalized.

TABLE 1: SCREENING OF SHORTLISTED PROTEIN EPITOPES BINDING TO MHC CLASS I ALLELE, INSTABILITY, ALIPHATIC INDEX AND GRAVY ANALYSIS

Epitope	Position	Score	Allele	Instability index	Result	Aliphatic index	GRAVY value
QLISAVNKL	76	2628.5007	HLA-A2	25.77	Stable	173.33	0.711
TMTVVVVSV	723	1576.6457	HLA-A2	5.69	Stable	161.11	2.3
GLVVAVAAL	1260	767.84646	HLA-A2	8.89	Stable	216.67	2.8
PLVPRNAEL	598	360.30828	HLA-A2	35.62	Stable	130	-0.122
WLQALIPLA	800	408.40239	HLA-A*0201	55.62	Unstable	195.56	1.5
NYPASHTTL	1231	360	HLA-A24	35.62	Stable	54.44	-0.689
RPQRQAGQL	66	360	HLA-A24	73.09	Unstable	54.44	-1.767
RNEATDGTL	396	360	HLA-A24	13.17	Stable	54.44	-1.244
KGRVVAIVL	254	300	HLA-A24	-9.98	Stable	194.44	1.544
CQIATNPVR	1101	400	HLA-A68.1	2.01	Stable	86.67	-0.089
GVGLVVAVA	1258	360	HLA-A68.1	-0.54	Stable	194.44	2.6
LVVAVAALI	1261	360	HLA-A68.1	8.89	Stable	260	3.344
NHMPADAER	430	240	HLA-A68.1	69.68	Unstable	22.22	-1.589
TRVVDAPSL	1130	200	HLA-B14	34.99	Stable	118.89	0.322
RRCITPYEL	752	300	HLA-B*2702	64.16	Unstable	86.67	-0.589
ARCPKGETL	461	200	HLA-B*2702	12.48	Stable	54.44	-0.722
YRAHTASAS	964	200	HLA-B*2702	-27.71	Stable	33.33	-0.656
IPEAAFTRV	1124	968	HLA-B*5101	25	Stable	97.78	0.533
SASAKLRVL	970	532.4	HLA-B*5101	20.86	Stable	141.11	0.6
AALILIVVL	1266	520	HLA-B*5101	20.86	Stable	303.33	3.6
TAECKDKNL	907	484	HLA-B*5101	48.28	Unstable	54.44	-1.211
GGVGLVVAV	1257	1100	HLA-B*5102	13.17	Stable	183.33	2.356
IFDNKGRVV	250	275	HLA-B*5201	39.48	Stable	107.78	-0.011
ITPEGAEEW	286	316.8	HLA-B*5201	38.29	Stable	54.44	-0.867
KGETLTVGF	465	240	HLA-B*5801	-18.36	Stable	75.56	0.133
YEKEPEETL	321	352	HLA-B60	77.44	Unstable	43.33	-1.967

TABLE 2: SCREENING OF SHORTLISTED PROTEIN EPITOPES BINDING TO MHC CLASS II ALLELE, INSTABILITY, ALIPHATIC INDEX AND GRAVY ANALYSIS

Epitope	Position	Score	Allele	Instability index	Result	Aliphatic index	GRAVY value	GRAVY value
YNMDYPPFG	1026	6.1	DRB1_0309	65.40	Unstable	0.00	-0.944	-0.944
LVGDKVMKP	161	5.6	DRB1_0309	-27.79	Stable	107.78	0.089	0.089
IVVLCVSFS	1270	5.7	DRB1_0423	-8.92	Stable	183.33	2.733	2.733
LQISFSTAL	1193	8.1	DRB1_0701	45.11	Unstable	141.11	1.211	1.211

IVLCNCLRL	810	7.7	DRB1_0701	39.06	Stable	205.56	1.900	1.900
VVSVATFIL	727	7.62	DRB1_0701	44.00	Unstable	194.44	2.667	2.667
VVAVAALIL	1261	7.6	DRB1_0701	30.29	Stable	260.00	3.344	3.344
VVVSVATFI	726	7.4	DRB1_0701	22.60	Stable	183.33	2.711	2.711
FKRSSKYDL	186	7.3	DRB1_0701	124.94	Unstable	43.33	-1.344	-1.344
IQVSLQIGI	405	6.9	DRB1_0701	27.30	Stable	205.56	1.478	1.478
LILIVVLCV	1267	6.5	DRB1_0701	12.48	Stable	313.33	3.944	3.944
VGFTDSRKI	470	6.4	DRB1_0701	32.82	Stable	75.56	-0.256	-0.256
LKIQVSLQI	403	6.3	DRB1_0701	17.87	Stable	205.56	1.011	1.011
YAVIRSMEF	27	6.1	DRB1_0701	84.02	Stable	86.67	0.567	0.567
VRTSAPCTI	442	5.6	DRB1_0701	58.13	Stable	86.67	0.522	0.522
WVMHKKEVV	667	5.4	DRB1_0701	89.41	Unstable	96.67	-0.100	-0.100
YGKNQVIML	635	5.3	DRB1_0701	-26.73	Stable	118.89	0.200	0.200
LIVVLCVSF	1269	5.2	DRB1_0701	-8.92	Stable	226.67	3.244	3.244
VLTVPTEGL	675	5.12	DRB1_0701	51.69	Unstable	151.11	1.011	1.011
LYPTMTVVV	719	5.12	DRB1_0701	19.40	Stable	140.00	1.556	1.556
FLLSLICCI	767	6.4	DRB1_0703	8.89	Stable	216.67	3.044	3.044
LTMRAVPQQ	83	5	DRB1_0806	64.71	Unstable	86.67	-0.233	-0.233
VRYKCNCGG	554	5	DRB1_0806	11.02	Stable	32.22	-0.533	-0.533
YELYPTMTV	717	5	DRB1_0817	11.02	Stable	75.56	0.089	0.089
IVNYPASHT	1228	5.2	DRB1_1501	26.19	Stable	86.67	-0.067	-0.067
IQVIRPRPR	57	6	DRB5_0101	26.56	Stable	118.89	-0.778	-0.778
VVLCVSFSR	1271	5.6	DRB5_0101	21.91	Stable	140.00	1.733	1.733
LRLLPCCCK	816	5.5	DRB5_0101	43.31	Stable	130.00	0.989	0.989

3.4. Antigenicity, Immunogenicity, and Allergenicity Prediction: The VaxiJen antigenicity likelihood predicted by ANTIGENpro was 0.4, indicating that the vaccine construct is antigenic. PLVPRNAEL score 1.1527, RNEATDGTL score 0.7553, CQIATNPVR scores 0.4220, and IFDNKGRVV score 0.7081 indicate that the construct is likely antigen for T cells. Similarly, the construct has a VGFTDSRKI score of 1.6420, VRYKCNCGG score of 2.1647, and IQVIRPRPR score of 1.0781 for Helper T lymphocytes was a good antigen.

The T lymphocyte epitopes NYPASHTTL score 0.3582, ARCPKGETL score -0.0872, YRAHTASAS score 0.3674, ITPEGAEEW scores 0.3800, and Helper T lymphocyte epitope IVNYPASHT -0.1663 were excluded from the research because they were non-antigen. PLVPRNAEL score 0.13807, RNEATDGTL score 0.18183, CQIATNPVR score 0.12904, YRAHTASAS score 0.00312 and ITPEGAEEW score 0.30998 were identified as immunogenic after extensive research. NYPASHTTL score -0.03944, ARCPKGETL score -0.09958, and IFDNKGRVV score -0.10961 were removed from the research due to their inability to obtain a positive score. CTL Epitopes PLVPRNAEL scores 0.7, RNEATDGTL scores 0.68, ARCPKGETL scores 0.68, and YRAHTASAS score 0.74 indicated that build by Allergen F. P., Non-Allergen. For Helper T lymphocyte VRYKCNCGG scores 0.69 and IVNYPASHT score 0.75, the construct was also likely Non-Allergen. NYPASHTTL score 0.74, CQIATNPVR scores 0.7, IFDNKGRVV score 0.72, ITPEGAEEW score 0.68, and Helper T lymphocyte epitopes VGFTDSRKI score 0.74, IQVIRPRPR score 0.68 were eliminated from the research as Allergens.

TABLE 3: PREDICTION OF ANTIGENICITY, IMMUNOGENICITY, AND ALLERGENICITY FOR MHC I

Epitope	Allele	Vaxijen score	Result	Immunogenicity	Allergenicity score	Prediction
PLVPRNAEL	HLA-A2	1.1527	Antigen	0.13807	0.7	Non Allergen
NYPASHTTL	HLA-A24	0.3582	Non antigen	-0.03944	0.74	Allergen
RNEATDGTL	HLA-A24	0.7553	Antigen	0.18183	0.68	Non Allergen
CQIATNPVR	HLA-A68.1	0.4220	Antigen	0.12904	0.7	Allergen
ARCPKGETL	HLA-B*2702	-0.0872	Non antigen	-0.09958	0.68	Non Allergen
YRAHTASAS	HLA-B*2702	0.3674	Non antigen	0.00312	0.74	Non Allergen
IFDNKGRVV	HLA-B*5201	0.7081	Antigen	-0.10961	0.72	Allergen
ITPEGAEEW	HLA-B*5201	0.3800	Non antigen	0.30998	0.68	Allergen

TABLE 4: PREDICTION OF ANTIGENICITY AND ALLERGENICITY FOR MHC II

Epitope	Allele	Vaxijen score	Result	Allergenicity score	Prediction
VGFTDSRKI	DRB1_0701	1.6420	Antigen	0.74	Allergen
VRYKCNCGG	DRB1_0806	2.1647	Antigen	0.69	Non Allergen
IVNYPASHT	DRB1_1501	-0.1663	Non antigen	0.75	Non Allergen
IQVIRPRPR	DRB5_0101	1.0781	Antigen	0.68	Allergen

3.5. B-cell epitope Prediction: All 120 epitopes predicted by ABCpred with a score greater than 0.5 were picked for each structural Protein. Predicted B-cell epitopes were used as a template for CTL and HTL epitopes. B cell epitopes overlapped were selected and chosen for the vaccine's final build.

TABLE 5: PREDICTION OF B CELL EPITOPE

Rank	Sequence	Start position	Score	Rank	Sequence	Start position	Score
1	AGRPGQFGDIQSRTPESK	933	0.94	15	PKARNPTVTYGKNQVIML	524	0.78
2	NVMRPGYYQLLQASLTCS	232	0.92	15	PDRTLMSQQSGNVKITVN	431	0.78
3	ELGDRKGKIHIPFPLANV	502	0.9	15	HMPPDTPDRTLMSQQSGN	425	0.78
4	NGDHAVTVKDAKFIVGPM	888	0.89	15	GARTALSVVTWNKDIVTK	165	0.78
4	VVSVATFILLSMVGMAAG	625	0.89	15	IREAEIEVEGNSQLQISF	1078	0.78
4	EIILYYYELYPTMTVVVV	609	0.89	15	KGKCAVHSMTNAVTIREA	1064	0.78
4	NCGGSNEGLTTTDKVINN	457	0.89	15	DIPEAAFTRVVDAPSLTD	1020	0.78
4	GIKTDDSHDWTKLRYMDN	310	0.89	16	MHKKEVVLTVPTEGLEVT	567	0.77
4	YYQLLQASLTCSPHRQRR	238	0.89	16	GEEPNYQEEWVMHKKEVV	556	0.77
5	DIQSRTPESKDVYANTQL	941	0.88	16	HIPFPLANVTCRVPKARN	511	0.77
6	GDKVMKPAHVKGTIDNAD	61	0.87	16	RCPKGETLTVGFTDSRKI	359	0.77
6	VTGYACLVGDKVMKPAHV	53	0.87	16	APCTITGTMGHFILARCP	344	0.77
6	NWHHGAVQYSGGRFTIPT	118	0.87	16	PTGAGKPGDSGRPIFDNK	134	0.77
7	YNMDYPPFGAGRPGQFGD	924	0.86	17	TAPFGCQIATNPVRAVNC	993	0.76
7	AKFIVGPMSSAWTPFDNK	898	0.86	17	NTQLSEAHVEKSESCKTE	839	0.76
7	TVIPSPYVKCCGTAECKD	792	0.86	17	AGMCMCARRRCITPYELT	641	0.76
7	TLAFLAVMSVGAHTVSAY	723	0.86	17	SHDWTKLRYMDNHMPADA	316	0.76
7	KWQYNSPLVPRNAELGDR	489	0.86	17	FTRVVDAPSLTDMSCEVP	1026	0.76
7	CGEGHSCHSPVALERIRN	277	0.86	18	RPGYSPMVLEMELLSVTL	760	0.75
7	TKDNFNVYKATRPYLAHC	257	0.86	18	HTVSAYEHVTVIPNTVGV	735	0.75
8	PVHMKSDASKFTHEKPEG	98	0.85	18	QYSGGRFTIPTGAGKPGD	125	0.75
8	FASAYRAHTASASAKLRV	857	0.85	18	ASHTTLGVQDISATAMSW	1131	0.75
8	IMLLYPDHPTLLSYRNMG	539	0.85	18	RAVNCAVGNMPISIDIPE	1006	0.75
8	SPHRQRRSTKDNFNVYKA	249	0.85	19	PAVGTVHVPYSQAPSGFK	963	0.74
9	PSGFKYWLKERGASLQHT	976	0.84	19	ESKDVYANTQLVLQRPAV	948	0.74
9	TLEPTLSLDYITCEYKTV	776	0.84	19	EKSESCKTEFASAYRAHT	848	0.74
9	DNHMPADAERAGLFVRTS	326	0.84	19	HAAVTNHKKWQYNSPLVP	481	0.74
9	QVSLQIGIKTDDSHDWTK	304	0.84	19	VALERIRNEATDGTLKIQ	287	0.74
9	TDGTLKIQVSLQIGIKTD	297	0.84	19	TGGVGLVVAVAALILIVV	1153	0.74
9	CAAECHPPKDHIVNYPAS	1115	0.84	20	NITVTAYANGDHAVTVKD	880	0.73
10	ANTQLVLQRPAVGTVHVP	954	0.83	20	VVTWNKDIVTKITPEGAE	172	0.73
10	VVYKGDVYNMDYPPFGAG	917	0.83	20	ATAMSWVQKITGGVGLVV	1143	0.73
10	DLECAQIPVHMKSDASKF	91	0.83	21	CTHPFHHDPPVIGREKFH	380	0.72
10	YKTLVNRPGYSPMVLEME	754	0.83	21	DMSCEVPACTHSSDFGGV	1037	0.72
10	VTWGNNEPYKYWPQLSTN	583	0.83	22	LQISFSTALASAEFRVQV	1091	0.71
10	TYGKNQVIMLLYPDHPTL	532	0.83	23	HTASASAKLRVLYQGNNI	864	0.69
10	TTDKVINNCKVDQCHAAV	467	0.83	23	PGATVPFLLSLICCIRTA	659	0.69
10	NVKITVNGQTVRYKCNCG	442	0.83	23	QVCSTQVHCAAECHPPKD	1107	0.69
10	IENDCIFEVKHEGKVTGY	39	0.83	24	RGASLQHTAPFGCQIATN	986	0.68
10	RRERMCMKIENDCIFEVK	31	0.83	24	PFMWGGAYCFCDAENTQL	825	0.68
10	AQKKKKPGRRERMCMKIE	23	0.83	24	ALIPLAALIVLCNCLRLL	700	0.68
11	ECKDKNLPDYSCKVFTGV	806	0.82	24	RRCITPYELTPGATVPFL	649	0.68
11	NEQQPLFWLQALIPLAAL	690	0.82	24	LLSYRNMGEEPNYQEEWV	549	0.68
11	HVKGTIDNADLAKLAFKR	69	0.82	24	RKISHSCTHPFHHDPPVI	374	0.68
11	LLSMVGMAAGMCMCARRR	633	0.82	24	DAERAGLFVRTSAPCTIT	332	0.68
12	TRPYLAHCPDCGEGHSCH	267	0.81	24	CSQPPCTPCCYEKEPEET	208	0.68

12	QKQQAPQNNTNQKKQPPK	2	0.81	24	GVQDISATAMSWVQKITG	1137	0.68
12	IVLGGANEGARTALSVVT	157	0.81	24	SMTNAVTIREAEIEVEGN	1071	0.68
12	SGRPIFDNKGRVVAIVLG	143	0.81	25	CCIRTAKAATYQEAAIYL	671	0.67
12	PPKDHIVNYPASHTTLGV	1121	0.81	25	VQSTAATTEEIEVHMPPD	412	0.67
13	PQLSTNGTAHGHPHEIIL	595	0.8	25	KELPCSTYVQSTAATTEE	404	0.67
13	EKFHSRPQHGKELPCSTY	394	0.8	25	PVMCLLANTTFPCSQPPC	196	0.67
13	TNQKKQPPKKKPAQKKKK	11	0.8	25	ACTHSSDFGGVAIIKYAA	1044	0.67
13	ALASAEFRVQVCSTQVHC	1098	0.8	26	TMGHFILARCPKGETLTV	351	0.66
13	FGGVAIIKYAASKKGKCA	1051	0.8	27	KLRVLYQGNNITVTAYAN	871	0.65
14	VIPNTVGVPYKTLVNRPG	745	0.79	27	YSCKVFTGVYPFMWGGAY	815	0.65
14	EVKHEGKVTGYACLVGDK	46	0.79	28	TKITPEGAEEWSLAIPVM	181	0.63
14	EWSLAIPVMCLLANTTFP	190	0.79	29	LTVGFTDSRKISHSCTHP	366	0.6
15	SAWTPFDNKIVVYKGDVY	907	0.78	30	LEMELLSVTLEPTLSLDY	768	0.52
15	LRLLPCCCKTLAFLAVMS	714	0.78	31	VGNMPISIDIPEAAFTRV	1012	0.51

3.6. Prediction of Shot listed Consensus Epitope: T cells regarded through B cell from those who had been not. PLVPRNAEL and RNEATDGTL epitopes were predicted as Consensus epitopes in my research based on their overlap with B cells.

3.7. Prediction of Toxicity, Hydrophobicity Hydropathicity, Hydrophilicity with Charge and Mol: Toxin Pred was used to assess toxicity and categories them as hazardous or harmless. A negative SVM score suggests that the epitopes chosen were not poisonous, hydrophobic, or hydropathic, but a positive SVM score implies that they should be hazardous. In my study, the epitopes PLVPRNAEL and RNEATDGTL had a negative SVM score, indicating that they were not toxic.

TABLE 6: PREDICTION OF TOXICITY, HYDROPHOBICITY, HYDROPATHICITY, HYDROPHILICITY WITH CHARGE AND MOL.WT

Epitope	SVM score	Prediction	Hydrophobicity	Hydropathicity	Hydrophilicity	Charge	Mol. Wt.
PLVPRNAEL	-1.09	Non-Toxin	-0.15	-0.12	0.07	0.00	1008.3
RNEATDGTL	-0.71	Non-Toxin	-0.35	-1.24	0.68	-1.00	976.13

3.8. Modeling, Refining, and Validation of Tertiary Structures: The method makes use of both PSIPRED's projected normal secondary structure information and Beta Turns' predicted information. The side-chain angles were taken by the rotamer library.

3.9. Structure-based Modeling and Evaluation of MHC Alleles: The 3D structure of the selected allele was created using the IPDIMGT/HLA allele Structure Prediction tool. The HLAA*24 and HLA A-2 alleles were modeled using MODELLER 9.10. Procheck was used to evaluate the stereochemical efficiency of a protein structure.

3.10. Prediction of IC_{50} Conservancy and Docking Analysis: After choosing less IC_{50} values, we were examined to see if epitomes were still conserved in the chikungunya virus proteome. Polyprotein component is the PLVPRNAEL Conservancy 99 percent and RNEATDGTL Conservancy 97 percent (Structural Protein) confirmed by the IEDB tool.

TABLE 7: PREDICTION OF SHORTLISTED CONSENSUS EPITOPES AND ANALYSIS OF IC_{50} VALUES, CONSERVANCY AND DOCKING ANALYSIS

Consensus epitopes	IC_{50} Value	Percentage of protein sequences identity	Minimum identity	Maximum identity	Docking score
PLVPRNAEL	HLA-A1 {0.693}, HLA-A2 {1.553}, HLA-A*02:01{0.35}, HLA-A*02:05{0.519}, HLA-A*11:01{.006}, HLA-A24 {1.792}, HLA-A3 {0.799}, HLA-A*31:01{1.609}, HLA-A*33:02{0.1}, HLA-A68.1 {1.609}, HLA-A2.1 {105.100}, HLA-B14{1.099},), HLA-B*27:05{2.996}, HLA-B*35:01{0.69}, HLA-B*37:01{0.405},	**99.00% (99/100)**	88.89%	100.00%	-244.051

RNEATDGTL	HLA B*38:01{0.445}, HLA-B*39:01{.3},HLA-B*39:02{0.693}, HLAB*44:03{0.405},HLAB*51:01{1.649}, HLAB*51:02{2.078}, HLA-B*52:01{0.884}, HLA-B*53:01{104}, HLA-B*54:01{113}, HLA-B*58:01{0.223}, HLA-B*7:02{116} HLA-A1 {0.693}, HLA-A2 [1.553}, HLA-A*02:01{1.143}, HLA-A*02:05{.005}, HLA-A*11:01{2.120}, HLA-A24 {1.792}, HLA-A3 {0.799}, HLA-A*31:01{0.12}, HLA-A*33:02{0.105}, HLA-A68.1 {1.609}, HLA-A2.1 {105.100}, HLA-B14{1.099}, HLA-B*27:05{35.5}, HLA-B*35:01{0.693}, HLA-B*27:02{0.15}, HLA-B*37:01{0.2}, HLA-B*52:01{0.25}, HLA-B*51:03{0.22}, HLA-B*51:02{.363}	97.00% (97/100)	88.89%	100.00%	-82.433

The success of Hpepdock docking generates a large number of findings, from which the top ten were selected for the study. After evaluating all ten docked conformations, the best-docked model was found to be number one, alleles HLA-A2, HLA-A24 bind to ligands PLVPRNAEL, RNEATDGTL Geometric form complexity docking scores of -244.051 and -82.433.

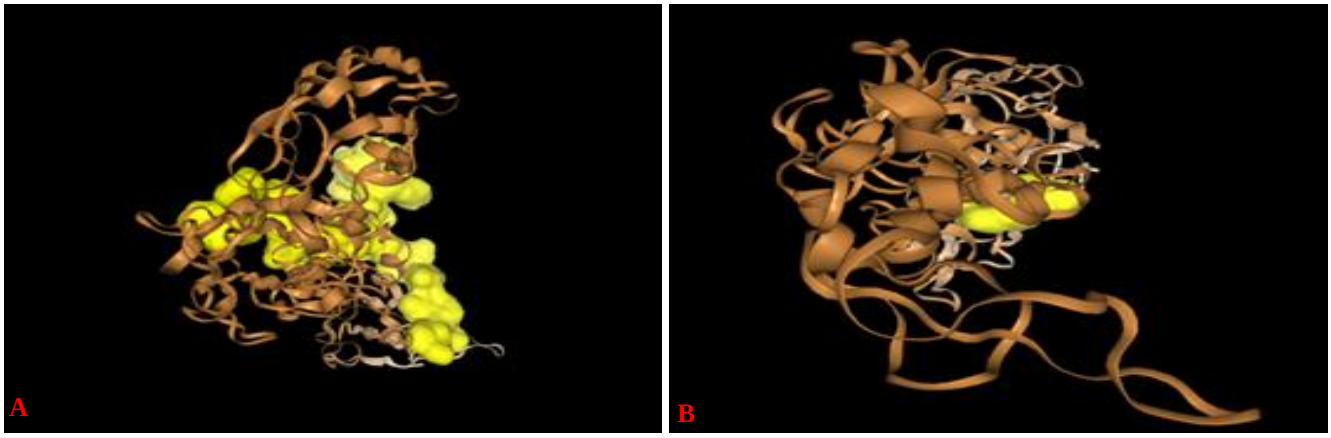

FIG. 1: EPITOPES (A) PLVPRNAEL INTERACTION WITH AN ANTIGEN-BINDING POCKET OF HLA-A2, EPITOPES (B) RNEATDGTL INTERACTION WITH AN ANTIGEN-BINDING POCKET OF HLA- A24

3.11. Population Coverage Analysis: With the aid of HLA binding and global coverage, each human fraction's response to specific epitope PLVPRNAEL covers 56.97 percent of the world population, whereas epitope RNEATDGTL covers 63.25 percent, according to my research.

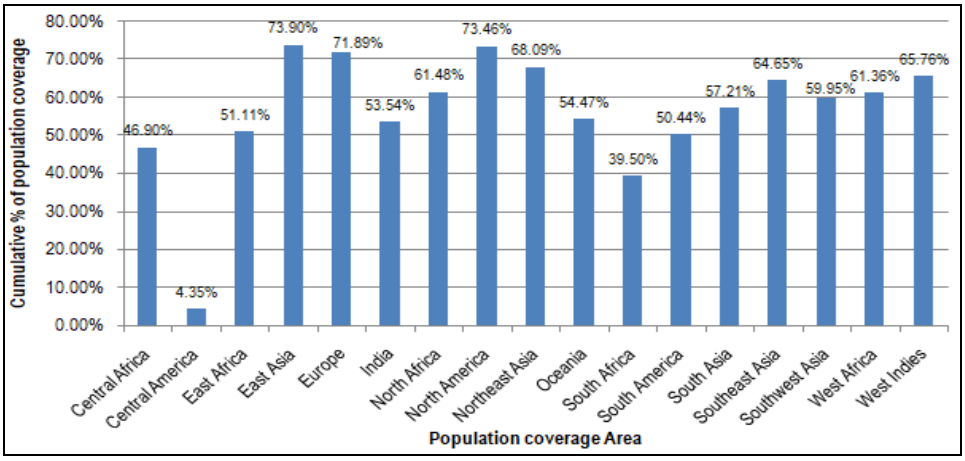

FIG. 2: POPULATION COVERAGE OF EPITOPE PLVPRNAEL

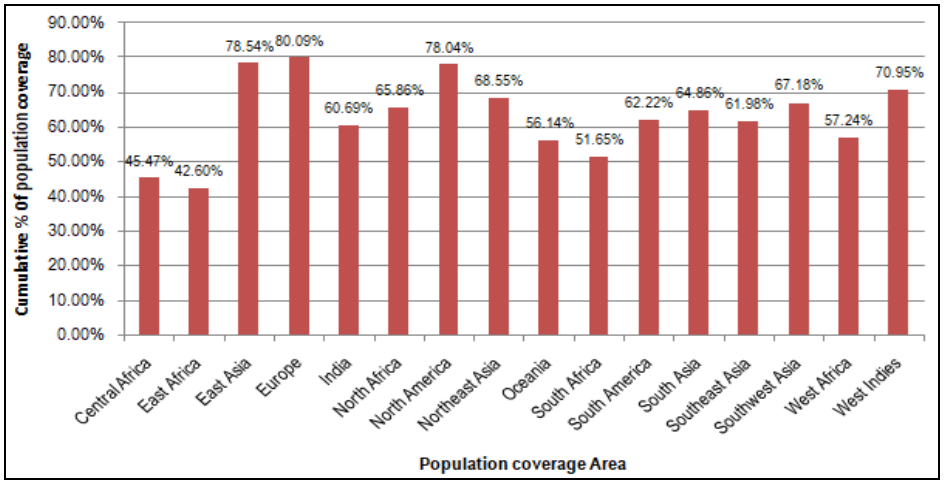

FIG. 3: POPULATION COVERAGE OF EPITOPE RNEATDGTL

DISCUSSION: In this work, the simulation algorithms and immunoinformatics top-down method to identify the Promiscuous T cell epitope and create a vaccine for chikungunya therapy. Researchers have used advances in immune-informatics techniques to identify potential antigenic epitopes from CHIKV proteins (especially the structural polyprotein) for peptide-based vaccine production [36].

The immune epitope database was used to predict nanomeric T cell epitopes, which was the first step in this research. The CHIKV structural polyprotein is essential for CHIKV entrance and fusion within human cells. As a result, structural polyprotein might be a promising target for inhibiting viral entry into the cell during the early stages. In my analysis, two T cell epitopes are shared with B cell linear epitope and have a high binding negative energy with the HLA class I allele.

Immunogenicity and antigenicity were determined after they were predicted. The modelled epitopes were docked with HLA class I alleles using the Hpepdock web service to better understand the structural analyses.

The structure of HLA-A2, HLA-A24 bind to PLVPRNAEL, RNEATDGTL Geometric form complexity docking scores of -244.051 and -82.433 were discovered by the authors of this work.

PLVPRNAEL and RNEATDGTL were determined to be much conserved. PLVPRNAEL and RNEATDGTL were shown to be the most promiscuous for chikungunya therapy in this investigation.

The epitopes PLVPRNAEL and RNEATDGTL were more immunogenic than previously reported peptides. Epitopes PLVPRNAEL and RNEATDGTL could be examined in the lab as CHIKV vaccine candidates. Different researchers conducted similar investigations on different viruses.

Pandey *et al.* (2018) 37 produced a multiepitope subunit vaccine utilising Zika virus structural and non-structural proteins using a combinatorial immunoinformatics technique Zika virus structural and non-structural proteins.

Using a mix of immunoinformatics and molecular docking, Shahid *et al.* (2020) [38] produced a MEBP vaccine. Using the ZIKV proteome, B-cell, T-cell and IFN-epitopes were predicted. Tahir ul Qamar *et al.* (2018) [39] used immunoinformatics and computational approaches to find conserved B and T cell epitopes on CHIKV structural proteins.

CONCLUSION: The viral epitopes PLVPRNAEL and RNEATDGTL interact with Protein that aids smooth conduction in the ER lumen and facilitates HLA class I allele binding. We analyze the sequencing, structure and conservation of CHIKV structural polyproteins, as well as homology modeling and docking. As a final result, epitopes PLVPRNAEL and RNEATDGTL will be used to treat chikungunya after lab confirmation.

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