IJPSR (2022), Volume 13, Issue 9 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 20 January 2022; received in revised form, 05 April 2022; accepted 26 April 2022; published 01 September 2022

DESIGN *IN-SILICO* MULTIPATHOGENIC VACCINE OF DENGUE AND ZIKA VIRUSES USING ENVELOPE PROTEIN

Neeraj Kumar Dixit

Department of Biotechnology, Saroj Institute of Technology & Management, Lucknow - 226002, Utter Pradesh, India.

Keywords:

MHC I and II, T-cell and B-cell epitopes, E-proteins, Dengue virus, Zika virus

Correspondence to Author:
Dr. Neeraj Kumar Dixit

Assistant Professor,
Department of Biotechnology,
Saroj Institute of Technology & Management, Lucknow - 226002,
Utter Pradesh, India.

E-mail: ndixitlip@gmail.com

ABSTRACT: The Flaviviridae family of viruses includes the dengue virus (DENV) and the Zika virus (ZIKV). Which have already caused outbreaks and epidemics in a number of countries in the entire world. Dengue fever and Zika fever are two and among the most widely disseminated mosquito-borne viral illnesses in the world both of these diseases have the potential to erupt in many places of the world at the same time, and they can be lethal as well as life-threatening. Unfortunately, there isn't a vaccine that works well enough to combat these viruses. As a result, we used an immunoinformatics method to build a multivalent and multipathogenic epitope-based vaccination that can combat both DENV and ZIKV infections at the same time in this study. ZIKV epitope QPENLEYRI and DENV epitope NKPTLDFEL docking scores of -346.10 and -379.80 were designed for a multivalent and multipathogenic vaccine that contained non-allergenic but also highly antigenic T-cell (100 percent conserved) from DENV and ZIKV serotypes.

INTRODUCTION: DENV and ZIKV virus are two flaviviruses spread by mosquitoes that affect approximately 1/2 of the worldwide population[1]. As a result of population expansion and migration, urbanization and climate change, Aedes aegypti disease has grown [2, 3, 4, 5]. The extrinsic incubation time for flaviviruses like DENV and ZIKV is predicted to be 10 to 14 days [6]. The composition of saliva varies when the salivary glands are infected, impacting blood acquisition and skin infection [7]. Infections with DENV, ZIKV and CHIKV stimulate the c-jun n terminal kinase (JNK) pathway, activating complement and apoptotic effectors and leading to a wide antiviral response [8].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(9).3622-34

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(9).3622-34

In contrast, whether administered as DNA, protein or produced by a chimpanzee adenovirus, ZIKV EDIII failed to suppress viral proliferation in mice [9, 10]. Adoptive transplantation of ZIKV-specific CD8 T cells decreased viral load in mice, [11, 12] indicating that Th1 cells are also necessary for full protection against ZIKV infection [13]. For both HLA classes, a variety of epitopes having strong binding affinity, promiscuous and antigenicity were predicted [14].

Controlled human challenge infection models are being researched as a potential alternative technique of obtaining effectiveness proof for vaccination regulatory clearance [15]. There is currently no effective Zika vaccine available; however, a ZIKV vaccine has been developed and is being evaluated in clinical trials [16, 17]. Sanofi Pasteur produced Dengvaxia, a licensed dengue vaccine that provides only limited protection and comes with a list of warnings [18, 19, 20]. It's also been licenced for usage in over 20 Countries where dengue fever is prevalent and the European Union

and the United State [21, 22]. Further investigation revealed that dengue sero youngsters under the age of 9 years had the lowest vaccination effectiveness of 14.4%. As a result, the goal of my research was to use computer simulations to discover potential vaccine candidates and antiviral efficacy testing. The goal of the study is to come up with safe and effective medicines for patients [23, 24]. Dengue virus infection can be symptomatic or asymptomatic depending on the serotype [25].

The febrile phase lasts two to seven days. Plasma leakage and hemorrhagic symptoms occur in DHF and Dengue Shock Syndrome DSS. Without correct treatment, severe plasma leakage can cause metabolic acidosis, shock, and organ damage in the foetus. In certain epidemiological research, secondary dengue virus infections were linked to an elevated risk of DHF/DSS [26]. During the early epidemics, patients reported ZIKV infection side effects Fever, angioedema, redness, and blindness are some of the symptoms, despite 50–80% of diseases being asymptomatic [27]. For the first time, Guillain-Barre syndrome was linked to Zika virus infection, indicating the virus's neurotropic nature; also, viral RNA was detected in semen and urine during this outbreak [28]. Autopsy samples were also used to confirm the foetus [29]. Since early 2016, the World Health Organization (WHO) has declared the Zika virus an international public health crisis is underway.

DENV and ZIKV both belong in Flavivirus; their genome organization and virion shape are identical. DENV and ZIKV have single-stranded positive RNA around 11, 000 nucleotides in ORF. DENV and ZIKV contain structural (C, prM and E) proteins with 7 (NS1, NS2A, NS2B, NS3, NS4A, NS4B NS5) non-structural proteins. In general, 55–56 percent of DENV and ZIKV polyproteins have similar sequences, but 69–72 percent of Dengue virus polyproteins are homologous in four serotypes. Non-structural proteins (NS1) are cytoplasmic proteins that play a role in viral RNA proliferation and polyprotein production. Each E dimer is made up of two antiparallel monomer units its helixes at the c-terminus are attached in the two-layer membrane of the virus E protein. Because E protein is immediately involved in the fusion of membrane and binding properties of the receptor after the entrance, it is largely exposed to the outside and hence contains the majority of neutralizing epitopes M proteins are buried in a pair beneath each dimeric E ectodomain in nooks and holes at the both E units on the viral membrane. Although antibodies against numerous viral proteins to a stimulus flavivirus infection here antibodies provide considerable protection and neutralize antibodies to the E protein on the viral surface [30].

Flavivirus envelope protein's ectodomain is made up of 3 subdomains [31]. II Domain is a large finger-like region that parallels to the virus surface and its tip contains a hydroxyl group. Domain III is an immune globulin molecule that can be folded projects out from the virus surface and is assumed to be crucial virus interaction. Domain I connects domains II and III with an eight-stranded barrel. E domain III was found to be the most common target DENV neutralizing antibodies, antibodies that area are primarily specific serotype [32]. While antibodies that the target fusion loops epitopes are typically have little neutralizing activity. On the contrary, some human investigations have directly demonstrated the relevance of ZIKV EDIII-specific antibodies. Antibodies against in a single study, E domain III with quaternary epitopes, were shown to be the best effective at neutralizing a panel for human mAb collected from various convalescent patients.

DENV and ZIKV's envelope proteins have a high amount of structural homology, with 35, 51, and 29 percent protein identity in EDI, EDII, and EDIII. Cross-reactivates within antibodies targeting two viruses' E domain I/II are common and less for antibodies targeting domain III. As a result, E protein is the best epitope-based vaccination target. The DNA vaccine comprising ZIKV prM and E protein developed by the National Institute of Allergy and Infectious Diseases and evaluated in a Phase II study, is the most advanced Zika vaccine candidate. Immunoinformatics is described as applying computational tools and methodologies for interpreting, creating and altering immunological knowledge [33]. Infections with DENV and ZIKV both induce potent T cell responses with unclear roles. Cross-reactive CD4 + T cells have been studied. The neutralization and enhancement activities of the dengue and Zika vaccines were both reduced, demonstrating that the

E dimeric structure on the top of VLP requires this space. Current preventative and treatment options for many infectious diseases are either inadequate or nonexistent. Although DENV and ZIKV have so many genetic and structural similarities, a nearly identical manner of transmission by a mosquito with the adaptive response, DENV and ZIKV are often confused. All of the aforementioned show that T cell and B cell responses are key components of adaptive immunity against dengue and Zika virus, that T cell and B cell components should be taken into account while developing protective vaccines. The present vaccine development is being guided by the application of immunoinformatics to the Antigens with As B and T cell epitope-based vaccination.

MATERIALS AND METHODS:

Viral Protein Selection: This study employed statistical approaches to predict the most successful DENV and ZIKV vaccine applications. The virus pathogen resource sequence database was used to retrieve the Dengue virus and Zika virus amino acid sequences. (https://www.viprbrc.org/brc/vipr-protein-serch.spg). DENV and ZIKV viral proteins were extracted using the FASTA format.

Predictions of Allergenicity and GRAVY of Protein: Allergen FP algorithm, which was mentioned in this study, was included (FP stands for Finger Print). The similarity index was used to select Envelope Proteins that aren't allergens [34]. Prot Param is software that calculates physical and chemical properties like stability for a protein in TrEMBLL and therefore user-entered epitope sequence. (http://www.expasy.org/sprot/) a comprehensive protein sequence database with high-quality annotations.

Prediction of MHC Epitopes from Zika and Dengue Vectors: ProPred1 is a web-based tool which anticipates MHC binding sites in antigenic protein sequences using a graphical interface. The server uses a matrix-based prediction technique. This site may be useful for detecting binding areas that are promiscuous in their ability to bind to a variety of HLA alleles. T-cell epitopes have crucial role for vaccine development, trigger immune response. The use of Propred1 for detecting T cell epitopes that bind with class I HLA alleles and increased affinity was studied [35].

The MHC Class II proteins, when combined with antigenic fragments, produce epitopes that T-helper cells (CD4+) detect. MHC Class II proteins are essential in almost every antigen reaction as a response. These proteins perform a direct or indirect function in a range of immune responses.

Prediction of Toxicity, Hydrophobicity Hydropathicity and Hydrophilicity: Epitope toxicity, hydrophobicity, hydropathicity, and hydrophilicity were all evaluated using the toxin Pred [36]. It can be used to locate the least cytotoxic peptides and to develop the least toxic peptides.

Prediction of Instability and GRAVY from Epitope: Expasy Prot Param calculated the index of Instability; hydropathicity with GRAVY. TrEMBL can be used to specify the epitope. The atomic and amino acid compositions are self-evident.

B-cell Epitope Prediction: The ABCpred service's purpose is to predict B cell epitope in an antigen sequence using an artificial neural network. This server is used for fixed-length patterns with a machine-based strategy. Use of a recurrent neural network, researchers was able to achieve an accuracy of 65.93 percent. The tabular outcome is a table that shows the length of amino acids in a protein predicted by the server from N-terminal to C-terminal [37].

2.7 Prediction of Short Listed Consensus Epitopes and Analysis of Antigenicity and Immunogenicity: Dengue and Zika Virus multivalent and multipathogenic vaccines could be developed using the prediction of shared epitopes between known serotypes. When the projected Zika and dengue virus serotype epitopes were evaluated, the standard peptides were discovered to be consensus epitopes.

The consensus epitope approach was used to filter out prospective candidates that were most likely to activate an immunological response against the Dengue Virus. Antigenicity is a characteristic of vaccines that decides whether an antigen or not. Epitope reacts to antibodies that lead the immune system to develop a defense mechanism against new threats. VaxiJen is the first server to forecast protective antigens without using alignment [38].

IEDB is an accessible tool that includes a comprehensive database for measuring experimentally immune epitopes as well as a set of tools. The IEDB includes tools for predicting and analysing the immunogenicity of epitopes [39].

Modeling, Refining and Validation of Tertiary Structures: PEPstr is a service that premises the tertiary structure of short peptides lengths ranging from 7 to 25 residues. This is a server that creates three-dimensional models of tiny peptide structures. This will allow researchers to develop modified peptides with the required therapeutic properties in mind [40, 41, 42].

Structure-Based Modeling of MHC Alleles and Evaluation: To prepare allele structures, the IMGT/HLA Database is used. As well as statistics on sequence validation Computational approaches were used to represent some of the allele structures that were not present in the IMGT/HLA database. Procheck is a programme that evaluates the stereo chemical quality of a protein structure in comparison to other well-refined structures [43, 44].

IC_{50} and Conservancy Analysis: Based on IC_{50} values, the prediction server Propred1 for each of the genes, we predicted the appropriate allele hypothesised T-cell epitopes. The IC_{50} value with the lowest value was chosen for further investigation.

Similarly, specific allele frequency was the sole variable that only measured tallied to meet the needs and demands of binding alleles for each amino acid. The redundant alleles were not taken into account. The IEDB's epitope conservancy research method to determine epitope conservation was utilised to forecast the target epitopes' conservancy trend .Conservancy can be calculated using provided identity factors, as well as the minimum and maximum conservancy amounts.

Analysis of Population Coverage: Tool IEDB was used to calculate the population coverage of the specified epitopes. Individuals of different ethnicities/countries, on the other hand, have distinct MHC-related pools/frequencies due to the significant polymorphism of MHC molecules. As a result, epitope-based vaccinations may be used to improve population distribution when reducing the amount of uncertainty and variation in coverage obtained or expected among ethnic groups.

Docking: Peptide protein interactions serve a critical role in immunological responses to name a few examples. A peptide sequence created by sampling probable peptide-binding conformations and using an energy scoring feature to score the anticipated protein-peptide complexes. HPEPDOCK is a tool that performs blind protein-peptide docking via the hierarchical method.

RESULTS:

Selection of Viral Proteins for Vaccine Preparation: The multivalent and multipathogenic vaccine was created using the virus pathogen resource sequence database. The structural envelope protein's amino acid sequence was determined using the dengue and zika viruses. The virus uses the structural protein to infiltrate the host and assemble viral particles. For this study, the E-proteins of DENV and ZIKV Flavivirus serotypes were chosen, and an epitope was created that elicited both B and T cell responses.

Predictions of Allergenicity and GRAVY of protein: The method presented in this work was made available on Allergen F P, a specially developed website. Non-allergenic Envelope proteins of DENV and ZIKV were chosen for further study. A higher negative score is determined as a result of the GRAVY analysis. Stability is determined using the similarity index, which includes physiochemical properties.

TABLE 1: PREDICTION OF ALLERGENICITY AND GRAVY OF PROTEIN

Name of vectors	predection score	Result	Molecular weight	Theoretical pI	Instability index	Classifies the protein	GRAVY value	Aliphatic index
Zeka	0.82	Non-allergen	54446.19	6.51	21.56	stable	-0.083	80.26
Dengue	0.82	Non-allergen	54200.72	7.91	27.73	stable	-0.082	84.81

Prediction of MHC Epitopes from ZIka and Dengue Xectors: Epitopes of Enevelope protein linked to the MHC class I allele were projecting from Propred I to classify DENV and ZENV CTL epitopes. The immunological acknowledgment is triggered by T-cell epitopes. A high rating demonstrates good precision. ZENV and DENV were predicted to have 30 and 16 CD8+ epitopes.

The Epitopes were stable with non-allergenic. Analysis' cutoff point was set at 4%, and epitope chose more than 100 values. The top 13 and 11 epitopes were picked from the Enevelope proteins bound to Helper T lymphocyte (HTL) that have MHC-II alleles of ZENV and DENV. The value cut-off is Actual Score 5 with 4 thresholds. As a result, more than 5 actual score epitopes will be included in the next study.

TABLE 2: SHORTLISTED MHC1 EPITOPES OF ZIKA AND TOXICITY, HYDROPHOBICITY, HYDROPATHICITY, HYDROPHILICITY, INSTABILITY AND GRAVY ANALYSIS

Epitope	Position	Score	Allele	SVM sore	Prede-ction	Hydro-phobicity	Hydro-pathicity	Hydro-philicity	Insta-bility index	Nature	(GRAVY) value
GLDFSDLYY	195	125	HLA-A1	-1.01	Non Toxin	0.02	-0.04	-0.49	14.22	stable	-0.044
GLFGKGSLV	106	1106	HLA-A2	-0.80	Non Toxin	0.15	0.97	-0.48	-9.98	stable	0.967
TMNNKHWLV	205	530	HLA-A2	-0.01	Non Toxin	-0.14	-0.64	-0.61	45.71	unstable	-0.644
ALGGVMIFL	490	270	HLA-A*0201	-0.97	Non Toxin	0.42	2.44	-1.24	22.60	stable	2.444
SYSLCTAAF	304	100	HLA-A24	-1.28	Non Toxin	0.11	1.01	-0.93	45.11	unstable	1.011
IVIGVGDKK	387	240	HLA-A68.1	-1.01	Non Toxin	-0.01	0.59	0.27	-19.41	stable	0.589
TVSNMAEVR	49	200	HLA-A68.1	-0.77	Non Toxin	-0.19	-0.09	0.13	22.60	stable	-0.100
CVTVMAQDK	30	120	HLA-A68.1	-0.68	Non Toxin	-0.12	0.33	0.00	16.76	stable	0.333
LVWLGLNTK	472	120	HLA-A68.1	-1.48	Non Toxin	0.07	0.62	-0.75	13.17	stable	0.689
GRLFSGHLK	282	2000	HLA-B*2705	-1.06	Non Toxin	-0.15	-0.28	-0.03	-0.54	stable	-0.311
HRSGSTIGK	401	2000	HLA-B*2705	-0.58	Non Toxin	-0.15	-1.02	0.39	48.28	unstable	-1.133
IRCIGVSNR	1	1000	HLA-B*2705	-0.19	Non Toxin	-0.22	0.20	0.04	8.89	stable	0.222
GRLITANPV	356	600	HLA-B*2705	-0.93	Non Toxin	-0.05	0.40	-0.31	27.09	stable	0.400
DPPFGDSYI	379	880	HLA-B*5101	-1.19	Non Toxin	-0.03	-0.54	-0.03	73.09	unstable	-0.600
DAHAKRQTV	247	242	HLA-B*5101	-0.43	Non Toxin	-0.42	-1.28	0.64	57.08	unstable	-1.278
QPENLEYRI	131	440	HLA-B*5102	-1.36	Non Toxin	-0.31	-1.31	0.35	30.81	stable	-1.456
KPTVDIELV	38	242	HLA-B*5102	-1.17	Non Toxin	-0.03	0.35	0.20	32.63	stable	0.389
TAAFTFTKV	309	220	HLA-B*5102	-1.71	Non Toxin	0.06	0.74	-0.57	13.17	stable	0.822
HAKRQTVVV	249	146.41	HLA-B*5103	-0.83	Non Toxin	-0.23	-0.14	0.03	66.51	unstable	-0.156
IPLPWHAGA	221	124.63	HLA-B*5301	-0.84	Non Toxin	0.17	0.42	-0.85	40.77	unstable	0.467
FSDLYYLTM	198	121.83	HLA-B*5301	-0.66	Non Toxin	0.08	0.47	-0.91	22.60	stable	0.522
PPFGDSYIV	380	121.56	HLA-B*5301	-0.96	Non Toxin	0.09	0.23	-0.48	63.66	unstable	0.256
WDFGSVGGV	429	183.58	HLA-B*5401	-0.99	Non Toxin	0.17	0.53	-0.62	4.79	stable	0.533
WFHDIPLPW	217	181.58	HLA-B*5401	-0.63	Non Toxin	0.14	-0.06	-1.04	23.89	stable	-0.067

FSQILIGTL	463	174.25	HLA-B*5401	-1.10	Non Toxin	0.22	1.40	-0.96	42.26	unstable	1.556
VPAQMAVDM	341	134.89	HLA-B*51	-1.03	Non Toxin	0.06	0.72	-0.34	48.70	unstable	0.800
KSLFGGMSW	454	264	HLA-B*5801	-1.07	Non Toxin	0.05	0.14	-0.60	71.42	unstable	0.144
KEWFHDIPL	215	139.18	HLA-B*0702	-0.73	Non Toxin	-0.07	-0.55	-0.10	-11.01	stable	-0.611
KLRLKGVSY	297	138.67	HLA-B*0702	-1.48	Non Toxin	-0.24	-0.30	0.19	1.99	stable	-0.333
RLKMDKLRL	292	138.34	HLA-B*0702	-0.94	Non Toxin	-0.51	-0.78	0.92	38.21	stable	-0.778

TABLE 3: SHORTLISTED MHC II EPITOPES OF ZIKA AND TOXICITY, HYDROPHOBICITY, HYDROPATHICITY, HYDROPHILICITY, INSTABILITY AND GRAVY ANALYSIS

Epitope	Position	Score	Allele	SVM score	Prede-ction	Hydro-phobicity	Hydro-pathicity	Hydro-philicity	Instability index	Nature	GRAVY value
IFLSTAVS	494	5.5	DRB1_0404	-1.38	Non-Toxin	0.22	1.64	-0.88	8.89	stable	1.856
YRIMLSVHG	136	6.7	DRB1_0405	-136	Non-Toxin	-0.02	0.47	-0.66	8.89	stable	1.856
MIFLSTAVS	494	5.5	DRB1_0410	-1.16	Non-Toxin	0.31	2.19	-1.19	22.60	stable	1.311
LGGFGSLGL	179	6.1	DRB1_0701	-1.05	Non-Toxin	0.29	1.31	-0.84	13.17	stable	2.433
LIGTLLVWL	466	5.8	DRB1_0701	-1.35	Non-Toxin	0.42	2.43	-1.59	42.26	unstable	0.244
FTKVPAETL	313	5.2	DRB1_0701	-1.45	Non-Toxin	-0.02	0.24	-0.12	-19.41	stable	0.700
VFNSLGKGI	436	5.1	DRB1_0701	-0.60	Non-Toxin	0.08	0.70	-0.46	-0.54	stable	0.856
LVTTTVSNM	44	5	DRB1_0701	-0.76	Non-Toxin	0.04	0.77	-0.68	28.41	stable	0.089
YVCKRTLVD	89	5.9	DRB1_0801	-0.89	Non-Toxin	-0.23	0.09	0.06	11.42	stable	0.011
LRLKGVSYS	297	5.1	DRB1_0801	-1.40	Non-Toxin	-0.16	0.01	-0.08	48.91	unstable	-1.000
WNNKEALVE	235	6.4	DRB1_0817	-0.66	Non-Toxin	-0.19	-0.90	0.22	8.89	stable	2.411
VMIFLSTAV	493	6.1	DRB1_1501	-1.18	Non-Toxin	0.30	2.17	-1.10	39.96	stable	0.067
VRGAKRMAV	414	5.4	DRB1_1501	-0.88	Non-Toxin	-0.29	0.07	0.41	8.75	stable	1.850

TABLE 4: SHORT LISTED MHC1 EPITOPES OF DENGUE AND TOXICITY, HYDROPHOBICITY, HYDROPATHICITY, HYDROPHILICITY, INSTABILITY AND GRAVY ANALYSIS

Epitope	Position	Score	Allele	SVM score	Prede-ction	Hydro-philicity	Hydro-pathicity	Hydro-philicity	Instability index	Nature	GRAVY value
NKPTLDFEL	37	474	HLA-A2	-1.36	Non-Toxin	-0.18	-0.70	0.30	18.44	stable	-0.700
LVLVGVVTL	479	186	HLA-A2	-1.32	Non-Toxin	0.41	3.01	-1.31	-9.98	stable	3.011
GMNSRSTSL	467	180	HLA-A2	-0.73	Non-Toxin	-0.27	-0.64	0.07	79.11	unstable	-0.644
FGSLGGVFT	422	291	HLA-A*0201	-1.21	Non-Toxin	0.26	1.21	-0.93	22.60	stable	1.211
VFTSIGKAL	428	110	HLA-A24	-1.06	Non-Toxin	0.14	1.26	-0.58	-0.54	stable	1.256
SVSLV	476	200	HLA-	-1.21	Non-	0.32	2.49	-1.00	-0.54	stable	2.489

LVGV			A68.1		Toxin						
TTMRGAKRM	404	180	HLA-A68.1	-0.63	Non-Toxin	-0.45	-1.01	0.57	17.87	stable	-1.011
RSTSLSVSL	471	126.6	HLA-A2.1	-1.19	Non-Toxin	-0.15	0.38	-0.14	57.71	unstable	0.378
TSLSVSLVL	473	124.1	HLA-A2.1	-1.38	Non-Toxin	0.19	1.86	-0.88	8.89	stable	1.856
SRSTSLSVS	470	300	HLA-B*2702	-1.14	Non-Toxin	-0.24	-0.13	0.09	79.11	unstable	-0.133
CPTQGEPSL	74	2000	HLA-B*2705	-1.41	Non-Toxin	-0.13	-0.64	0.03	56.33	unstable	-0.644
MDLEKRHVL	340	480	HLA-B*4403	-1.20	Non-Toxin	-0.30	-0.54	0.57	66.51	unstable	-0.544
NSRSTSLSV	469	286	HLA-B*5101	-0.91	Non-Toxin	-0.28	-0.43	0.08	79.11	unstable	-0.433
LVLVGVVTL	479	484	HLA-B*5102	-1.32	Non-Toxin	0.41	3.01	-1.31	-9.98	stable	3.011
FKNPHAKKQ	240	183.58	HLA-B*5401	-0.12	Non-Toxin	-0.47	-2.10	0.66	15.30	stable	-2.100
AKKQDVVVL	245	136.95	HLA-B*0702	-0.89	Non-Toxin	-0.13	0.38	0.27	56.60	stable	0.378

TABLE 5: SHORTLISTED MHC II EPITOPES OF DENGUE AND TOXICITY, HYDROPHOBICITY, HYDROPATHICITY, HYDROPHILICITY, INSTABILITY AND GRAVY ANALYSIS

Epitope	Position	Score	Allele	SVM score	Prede-ction	Hydro-phobicity	Hydro-pathicity	Hydro-philicity	Instability index	Nature	GRAVY
LRMDKLQLK	286	5.8	DRB1_0301	-1.30	Non-Toxin	-0.35	-0.60	0.55	8.75	stable	1.850
LVGVVTLYL	480	5	DRB1_0410	-1.05	Non-Toxin	0.32	2.16	-1.26	-21.42	stable	0.467
LVLVGVVTL	478	8.1	DRB1_0701	-1.28	Non-Toxin	0.37	2.71	-1.18	8.89	stable	1.856
IQMSSGNLL	269	7.3	DRB1_0701	-0.89	Non-Toxin	0.04	0.50	-0.57	22.60	stable	1.311
MKILIGVVI	454	5.2	DRB1_0701	-1.71	Non-Toxin	0.31	2.33	-0.85	13.17	stable	2.433
WLVHRQWFL	205	5.3	DRB1_0703	-0.45	Non-Toxin	0.01	0.18	-1.30	42.26	unstable	0.244
LIGVVITWI	457	5	DRB1_0703	-1.28	Non-Toxin	0.42	2.37	-1.40	-19.41	stable	0.700
FVCKHSMVD	89	6.6	DRB1_0817	-0.31	Non-Toxin	0.42	0.47	-0.22	-7.81	stable	0.467
WIQKETLVT	230	5.4	DRB1_0817	0.72	Non-Toxin	-0.06	-0.07	-0.3	-0.54	stable	-0.078
MRGAKRMAI	405	5.8	DRB1_1501	-1.20	Non-Toxin	-0.30	-0.16	0.40	11.42	stable	0.011
LVTFKNPHA	236	5.5	DRB1_1501	-0.79	Non-Toxin	-0.05	-0.03	-0.40	48.91	unstable	-1.000

Prediction of Toxicity, Hydrophobicity, Hydropathicity and Hydrophilicity: The Toxin Pred program was used to test toxicity, which classed them toxic or non-toxic. A negative SVM score shows that the chosen epitopes were non-toxic, hydrophobic, and hydropathic, while a positive SVM score indicates that they should be studied further.

Prediction of Instability and GRAVY from Epitopes: Prot Param is a carefully curated protein sequence database with high-quality annotations. This approach was used to choose research with higher GRAVY negative scores and stability. We discovered 8 MHC1 and 0 MHC II epitopes for the Zika virus and 3 MHC I and 1 MHC II epitopes for the Dengue virus with stable protein, which we utilized in the next investigation.

Prediction of B-cell Epitope: For envelope protein of ZENV and DENV, all epitopes expected via ABC pred with a score greater than 0.50 have been chosen. For ZENV and DENV, 49 and 54 B. cell epitopes have been projected from envelope proteins, respectively.

In the design of the final vaccine, projected B-cell epitopes were utilized as a basis for CTL and HTL epitopes. B cell epitopes overlapped with T cells were selected and accepted for inclusion in vaccine's final design.

TABLE 6: PREDICTION OF SHORTLISTED B CELL EPITOPES FROM ZIKA AND DENGUE VECTORS

ZEKA				DENGUE			
Rank	**Sequence**	**Start position**	**Score**	**Rank**	**Sequence**	**Start position**	**Score**
1	TVEVQYAGTDGPCKVP	327	0.94	1	HGTIVIRVQYEGDGSP	317	0.94
1	AKVEVTPNSPRAEATL	165	0.94	1	YGTVTMECSPRTGLDF	178	0.94
2	TGHETDENRAKVEVTP	156	0.93	2	KYCIEAKLTNTTTASR	58	0.90
3	FGSLGLDCEPRTGLDF	183	0.91	2	PWLPGADTQGSNWIQK	219	0.90
4	SGMIVNDTGHETDENR	149	0.89	3	KGMSYSMCTGKFKVVK	295	0.89
5	AVLGDTAWDFGSVGGV	422	0.88	3	ECSPRTGLDFNEMVLL	184	0.89
5	TVMAQDKPTVDIELVT	32	0.88	4	TTMAKNKPTLDFELIK	32	0.88
5	EWFHDIPLPWHAGADT	216	0.88	4	KGGIVTCAMFTCKKNM	110	0.88
6	YEASISDMASDSRCPT	61	0.87	5	TTASRCPTQGEPSLNE	69	0.87
6	KFTCSKKMTGKSIQPE	118	0.87	5	IGVVITWIGMNSRSTS	459	0.87
7	GDKKITHHWHRSGSTI	392	0.86	5	GVSWTMKILIGVVITW	450	0.87
7	YSLCTAAFTFTKVPAE	305	0.86	6	DSYIIIGVEPGQLKLS	375	0.86
7	SGHLKCRLKMDKLRLK	286	0.86	6	TGHLKCRLRMDKLQLK	280	0.86
7	PWHAGADTGTPHWNNK	224	0.86	7	AILGDTAWDFGSLGGV	413	0.84
7	GGTWVDVVLEHGGCVT	17	0.86	7	FETTMRGAKRMAILGD	402	0.84
8	RGWGNGCGLFGKGSLV	99	0.85	7	GSWVDIVLEHGSCVTT	18	0.84
9	GRLITANPVITESTEN	356	0.84	8	QSSITEAELTGYGTVT	167	0.83
10	IELVTTTVSNMAEVRS	43	0.83	8	TIVITPHSGEENAVGN	138	0.83
10	NPVITESTENSKMMLE	362	0.83	9	HSMVDRGWGNGCGLFG	94	0.82
10	DFSDLYYLTMNNKHWL	197	0.83	9	SSIGQMFETTMRGAKR	396	0.82
11	RGAKRMAVLGDTAWDF	416	0.82	9	GSNWIQKETLVTFKNP	228	0.82
12	GVSNRDFVEGMSGGTW	5	0.81	10	VLEHGSCVTTMAKNKP	24	0.81
12	GMSWFSQILIGTLLVW	459	0.81	10	AMFTCKKNMEGKIVQP	117	0.81
12	KSIQPENLEYRIMLSV	128	0.81	11	AEPPFGDSYIIIGVEP	369	0.79
13	GEAYLDKQSDTQYVCK	78	0.8	11	GRLITVNPIVTEKDSP	349	0.79
13	ASDSRCPTQGEAYLDK	69	0.8	11	PFEIMDLEKRHVLGRL	336	0.79
13	MMLELDPPFGDSYIVI	374	0.8	12	KTEAKQPATLRKYCIE	47	0.78
13	AGTDGPCKVPAQMAVD	333	0.8	12	FGAIYGAAFSGVSWTM	440	0.78
14	GSTIGKAFEATVRGAK	404	0.79	12	NPIVTEKDSPVNIEAE	355	0.78
14	VLEHGGCVTVMAQDKP	24	0.79	13	AVGNDTGKHGKEVKVT	150	0.77
15	YVCKRTLVDRGWGNGC	90	0.78	14	KIVQPENLEYTIVITP	128	0.75
15	EALVEFKDAHAKRQTV	240	0.78	15	QMENKAWLVHRQWFLD	200	0.74
15	FVEGMSGGTWVDVVLE	11	0.78	16	FTSIGKALHQVFGAIY	429	0.73
16	DMQTLTPVGRLITANP	348	0.77	16	TGATEIQMSSGNLLFT	265	0.73
16	RAEATLGGFGSLGLDC	175	0.77	16	KHGKEVKVTPQSSITE	157	0.73
16	SVHGSQHSGMIVNDTG	142	0.77	16	MRCIGISNRDFVEGVS	1	0.73
17	KVPAQMAVDMQTLTPV	340	0.76	17	HSGEENAVGNDTGKHG	144	0.71

18	GDSYIVIGVGDKKITH	383	0.73	18	ETLVTFKNPHAKKQDV	235	0.70
19	TVSNMAEVRSYCYEAS	49	0.71	19	QDVVVLGSQEGAMHTA	248	0.69
19	EMDGAKGRLFSGHLKC	276	0.71	20	GSQEGAMHTALTGATE	254	0.68
19	HWLVHKEWFHDIPLPW	210	0.71	21	NRDFVEGVSGGSWVDI	8	0.66
20	QILIGTLLVWLGLNTK	465	0.7	21	QGEPSLNEEQDKRFVC	77	0.66
20	LGKGIHQIFGAAFKSL	441	0.7	21	KPTLDFELIKTEAKQP	38	0.66
21	NGSISLTCLALGGVMI	481	0.69	21	RVQYEGDGSPCKIPFE	323	0.66
22	IFGAAFKSLFGGMSWF	448	0.66	22	KEIAETQHGTIVIRVQ	310	0.65
23	AGALEAEMDGAKGRLF	270	0.65	23	KLSWFKKGSSIGQMFE	388	0.64
24	TFTKVPAETLHGTVTV	313	0.62	23	VEPGQLKLSWFKKGSS	382	0.64
24	GSQEGAVHTALAGALE	259	0.62	24	NGCGLFGKGGIVTCAM	103	0.63
25	DAHAKRQTVVVLGSQE	247	0.54	25	WDFGSLGGVFTSIGKA	420	0.61
				26	MCTGKFKVVKEIAETQ	301	0.60
				27	IGMNSRSTSLSVSLVL	466	0.59
				28	NEEQDKRFVCKHSMVD	83	0.58
				29	DFNEMVLLQMENKAWL	192	0.54
				30	NPHAKKQDVVVLGSQE	242	0.51

Prediction of Consensus Epitope, Antigenicity and Immunogenicity: We created peptide datasets with equal MHC binding affinity and separated those that were and weren't recognized by T cells. In my research, I projected a total of 7 consensus epitopes. DENV and ZIKV were projected to have 4, 3 epitopes. Consensus B cell epitopes were chosen from among B cell epitopes that were antigenic to anticipate T cell epitopes. Epitopes RLKMDKLRL, QPENLEYRI, GRLFSGHL had a score of 0.86, 0.81, and 0.71 for Zika and NKPTLDFEL, TTMRGAKRM, FKNPHAKKQ and WIQKETLVT score 0.88, 0.84, 0.70 and 0.82 for Dengue Consensus B cell epitopes were selected in each case. Antigenicity Epitopes GRLFSGHL, RLKMDKLRL score-1.3059, -1.3693 of ZIKAV were ruled out for future study. Similarly, epitopes TTMRGAKRM, FKNPHAKKQ, and WIQKETLVT with DENV antigen scores of 0.1214, -0.2365, and 0.2331 were ruled out for future study due to non-antigen. For the next study, only epitope QPENLEYRI, NKPTLDFEL score 1.2917, 0.6084 of ZIKV, and DENV as an antigen were used. T cells with a high Immunogenicity score are more likely to be recognized, while those with a low Immunogenicity score are less likely to be accepted. The epitope RLKMDKLRL -0.40722 of ZIKAV was eliminated for further analysis after failing to obtain a positive value. Similarly, DENV epitopes TTMRGAKRM, FKNPHAKKQ, and WIQKETLVT with scores of -0.08685, -0.25293, and -0.1058, respectively, were rejected for further analysis. The epitopes of ZIKV and DENV that QPENLEYRI, GRLFSGHL, and NKPTLDFEL score 0.13656, 0.0123, and 0.20284 generate a positive score were chosen for the next study. The antigenicity and immunogenicity of the vaccine constructs were QPENLEYRI and NKPTLDFEL, showing that it is both antigenic and immunogenic. Both epitopes imply that the final vaccine design was a potent antigen as a result of this approach, which was chosen for further research.

TABLE 7: PREDICTION OF SHORTLISTED CONSENSUS EPITOPES AND ANALYSIS OF ANTIGENICITY AND IMMUNOGENICITY

Types of vectors	Epitopes	Start position	Score	Protective score	Probability of Antigenicity	Immunogenicity
Zika	KSIQPENLEYRIMLSV	128	0.8	1.2917	ANTIGEN	0.13656
	EMDGAKGRLFSGHL	276	0.71	-1.3059	NON-ANTIGEN	
	SGHLKCRLKMDKLRLK	286	0.86	-1.3693	NON-ANTIGEN	
Dengue	TTMAKNKPTLDFELIK	32	0.88	0.6084	ANTIGEN	
	FETTMRGAKRMAILG	402	0.84	0.1214	NON-ANTIGEN	
	ETLVTFKNPHAKKQDV	235	0.70	-0.2365	NON-ANTIGEN	
	GSNWIQKETLVTFKNP	228	0.82	0.2331	NON-ANTIGEN	-0.1058

Tertiary Structure Modeling, Refinement, and Evaluation: PEPstr is a program that allows you to run lengthy simulations of predicted peptides. This will allow researchers to develop modified peptides with the required therapeutic properties in mind. PSIPRED's projected standard secondary structure

information and Beta Turns' anticipated information are both used in the procedure.

Allele Structure: The 3D structure of the given allele HLA B*2705 was built using the IPD IMGT/HL allele Structure Prediction service (2BSR). MODELLER 9.10 is homology simulation programme that the model picked with allele HLA A-2 as Sample Template PDB ID (4U6X).PROCHECK was used to check the allele's stereo chemical properties.

Docking: Hpepdock online server, which was utilized for protein docking. Hpepdock docking's success provides a huge number of results, from which the top ten were chosen for analysis. After examining all ten docked conformations, the best-docked model was number one, demonstrating the best interactions between the receptor and ligand. ZIKV and DENV alleles HLA-B*2705, HLA-A2 bind to QPENLEYRI, NKPTLDFEL. Geometric form complexity docking scores of -346.10 and -379.80 were discovered.

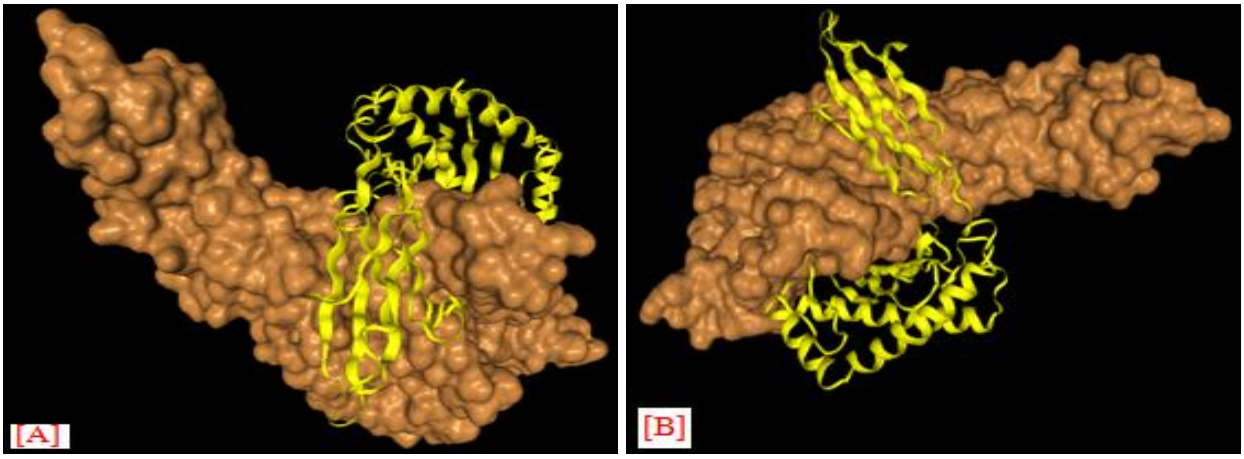

FIG. 1: EPITOPES (A) QPENLEYRI (ZIKA) INTERACTION WITH AN ANTIGEN-BINDING POCKET OF HLA-B*27: 05 EPITOPES (B) NKPTLDFEL (DENGUE) INTERACTION WITH AN ANTIGEN-BINDING POCKET OF HLA-A2

IC_{50} Values and Conservancy Prediction through Consensus Sequences: In the Informatics in Medicine Unlocked 20 (2020) 1004306 IEDB, Alleles having the ideal IC50 values were chosen the ideal binders to investigate next study. E (Structural Protein) component was the QPENLEYRI and NKPTLDFEL Conservancy 100 percent of ZIKV and DENV confirmed by the IEDB tool.

TABLE 8: PREDICTION OF IC 50 VALUES, CONSERVANCY ANALYSIS AND DOCKING

Types of vectors	Epitope sequence	IC 50 Value	Percentage of protein sequences that match with 100% identity	Minimum identity	Maximum identity	Docking score
Zika	QPENLEYRI	HLA-B*35:01{0.693}, HLA-B*37:01{0.405}, HLA, B*38:01{0.445}, HLA-B*39:01{1.7920}, HLA-B*39:02{0.693}, HLAB*44:03{0.405}, HLAB*51:01{1.649}	**100.00% (55/55)**	100.00%	100.00%	-346.10
Dengue	NKPTLDFEL	HLA-A1 {0.693}, HLA-A2 {1.553}, HLA-A*02:01{1.143}, HLA-A*02:05{0.519}, HLA-A*11:01{-2.120}, HLA-A24 {1.792}, HLA-A3 {-0.799}, HLA-A*31:01{1.609}, HLA-A*33:02{0.105}, HLA-A68.1 {1.609}, HLA-A2.1	100.00% (69/69)	100.00%	100.00%	-379.80

Population Coverage Study: The selection of a group of epitopes with a large number of HLA binding capacities will aid in the expansion of global coverage. We have a propensity to be ready to develop the response of every human fraction to a specified epitope by utilizing HLA constitution frequencies. In my research, I discovered that the ZIKV epitope QPENLEYRI covers 53.49 % of the entire world's population while the DENV epitope NKPTLDFEL covers 54.31 % of the entire world's population.

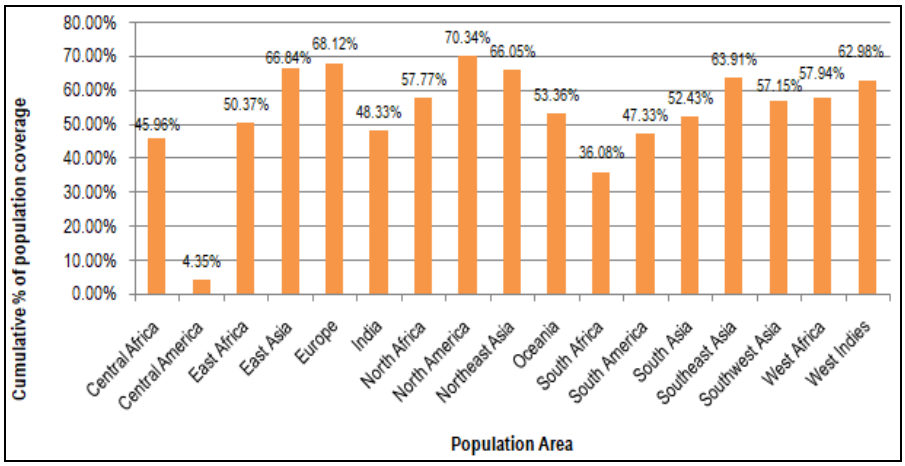

FIG. 2: POPULATION COVERAGE OF EPITOPE (ZIKV) QPENLEYRI

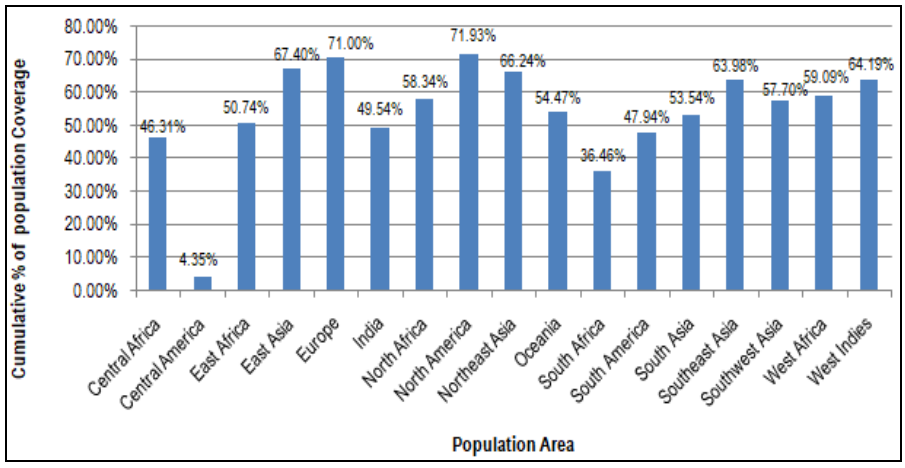

FIG. 3: POPULATION COVERAGE OF EPITOPE (DENV) NKPTLDFEL

DISCUSSION: We presented a potential epitopes QPENLEYRI, NKPTLDFEL of ZIKV and DENV based on this information; it fights and defeats Dengue and Zika viruses by a mixture of immunizations and vector blockage. In order to anticipate epitope vaccine candidate agents against virus, immunoinformatics methods are becoming increasingly popular. We found consecutive amino acids when screening, so this whole ZIKV and DENV proteome included THL, CTL, and B cell epitopes. The use of immunoinformatics method epitope cluster will pave the way for further research for the development of a ZIKV and DENV synthetic epitope vaccination that is precisely targeted. The rapid Zika virus outbreaks have added to the difficulty of solving the dengue problem. Like any other scientific challenge before it, the enormous global outbreaks of ZIKV and DENV have prompted substantial research into flavivirus virology, immunology, and vaccinology.

CONCLUSION: The predicted immunogenic epitopes QPENLEYRI, NKPTLDFEL of ZIKV, and DENV were docked with the most common MHC molecules observed in our studies HLAB*27:05, HLA A-2. Two-pronged approaches combine human preventive vaccines with vector blockade to interrupt transmission cycles in several points required to tackle mosquito-borne viruses. B cell and T cell vaccinations are anticipated aid in overcoming the impact on humans, prevent mosquito transmission, and allow for

comprehensive disease control. However, more experimental testing is required to determine these procedures' practicality, efficiency, and safety.

ACKNOWLEDGEMENT: We would like to acknowledge the Department of Biotechnology, Saroj Institute of Technology & Management, Lucknow, Utter Pradesh, India, for the infrastructure support.

CONFLICTS OF INTEREST: There are no conflicts of interest declared by the authors.

REFERENCES:

1. Kraemer MUG, Reiner RCJR and Brady OJ: Past and future spread of the arbovirus vectors Aedes aegypti and Aedes albopictus. Nat Microbiol 2019; 4: 854-863.
2. Gutiérrez-Bugallo G, Piedra LA and Rodriguez Ml: Vector-borne transmission and evolution of Zika virus. Nat Ecol Evol 2019; 3: 561-569.
3. Kraemer MUG, Reiner RC and Brady OJ: Past and future spread of the arbovirus vectors Aedes aegypti and Aedes albopictus. Nat Microbio 2019; 4: 854-863.
4. Brady OJ and Hay SI: The first local cases of Zika virus in Europe. Lancet.2019; 394: 1991–2.
5. Philip C, Novick CG and Novick LF: Local Transmission of Zika Virus in Miami-Dade County: The Florida Department of Health Rises to the Challenge. J Public Health Management Practice 2019; 25: 277 87.
6. Winokur OC, Main BJ, Nicholson J and Barker CM: Impact of temperature on the extrinsic incubation period of Zika virus in Aedes aegypti 2020; 14: 0008047.
7. Guerrero D, Cantaert T and Missé D: Aedes mosquito salivary components and their effect on the immune response to arboviruses. Front Cell Infect Microbiol 2020; 10: 407.
8. Chowdhury A: JNK pathway restricts DENV2, ZIKV and CHIKV infection by activating complement and apoptosis in mosquito salivary glands 2020; 16: 1008754.
9. Wang X: Effects of adjuvants on the immunogenicity and efficacy of a Zika Virus envelope domain III subunit vaccine. Vaccines 2019; 7(4): 161.
10. López-Camacho and César: Immunogenicity and Efficacy of Zika Virus Envelope Domain III in DNA, Protein, and ChAdOx1 Adenoviral-Vectored Vaccines 2020; 8(2): 307.
11. Lucas CGO, Kitoko JZ and Ferreira FM: Critical role of CD4+ T cells and IFNγ signaling in antibody-mediated resistance to Zika virus infection. Nat Commun 2018; 9: 313.
12. Koblischke M: Structural influence on the dominance of virus-specific CD4 T cell epitopes in Zika virus infection. Front. Immunol 2018; 9: 01196
13. Elong Ngono A and Shresta S: Cross-reactive T cell immunity to dengue and Zika viruses. New Insights into Vaccine Development 2019; 10: 1316.
14. Gillespie GM: Functional heterogeneity and high frequencies of cytomegalovirus-specific CD8 (+) T lymphocytes in healthy seropositive donors. J. Virol 2000; 74(17): 8140–8150.
15. Vannice KS, Cassetti MC and Eisinger RW: Demonstrating vaccine effectiveness during a waning epidemic a WHO/NIH meeting report on approaches to development and licensure of Zika vaccine candidates. Vaccine 2019; 37: 863-868.
16. Young G: Complete protection in macaques conferred by purified inactivated Zika vaccine: defining a correlate of protection. Sci Rep 2020; 10: 3488.
17. Han HH: Safety and immunogenicity of a purified inactivated Zika virus vaccine (PIZV) candidate in healthy adults a randomized, observer-blind phase 1 clinical trial. Lancet Infect. Dis 2021; 21: 1282–1292.
18. Whitehead SS and Subbarao K: Which dengue vaccine approach is the most promising, and should we be concerned about enhanced disease after vaccination: the risks of incomplete immunity to dengue virus revealed by vaccination. Cold Spring Harb 2018; 10(6): 028811.
19. Coronel MDJ, Park J and López E: Immunogenicity and safety of simplified vaccination schedules for the CYD-TDV dengue vaccine in healthy individuals aged 9-50 years (CYD65) a randomised, controlled, phase 2, non-inferiority study, The Lancet Infectious Diseases 2021; 21(4): 517–528.
20. Deng SQ, Yang X, Wei Y, Chen JT, Wang XJ and Peng HJ: A review on dengue vaccine development Vaccines 2020; 8(1): 63.
21. World Health Organization [WHO]. Dengue vaccine: WHO position paper, July 2016 - recommendations. Vaccine 2017; 35: 1200–1201.
22. Halstead SB and Dans LF: Dengue infection and advances in dengue vaccines for children. Lancet Child Adolesc Health 2019; 3: 734–741.
23. Pushpakom S, Iorio F, Eyers PA, Escott KJ, Hopper S, Wells A, Doig A, Guilliams T, Latimer J, McNamee C, Norris A, Sanseau P, Cavalla D and Pirmohamed M: Drug repurposing: progress, challenges and recommendations. Nat Rev Drug Discov 2018; 18: 41-58.
24. Aguiar M and Stollenwerk N: Dengvaxia age as surrogate for serostatus. Lancet Infect Dis 2018; 18(3): 245.
25. John AL and Rathore APS: Adaptive immune responses to primary and secondary dengue virus infections. Nat Rev Immunol 2019; 19: 218–230.
26. Katzelnick LC, Gresh L, Halloran ME, Mercado JC, Kuan G and Gordon A: Antibody-dependent enhancement of severe dengue disease in humans. Science 2017; 358: 929–932.
27. Weaver SC, Costa F, Garcia-Blanco MA, KO AI, Ribeiro G S and Saade G: Zika virus: History, emergence, biology, and prospects for control. Antiviral Res 2016; 130: 69–80.
28. Musso D, Bossin H, Mallet HP, Besnard M, Broult J and Baudouin L: Zika virus in French Polynesia 2013-14: anatomy of a completed outbreak. Lancet Infect Dis 2018; 18: 172-182.
29. Mlakar J, Korva M, Tul N, Popovic M, Poljsak-Prijatel j M and Mraz J: Zika virus associated with microcephaly. N Engl J Med 2016; 374: 951–958.
30. Stettler K, Beltramello M, Espinosa DA, Graham V, Cassotta A and Bianchi S: Specificity, cross-reactivity, and function of antibodies elicited by Zika virus infection. Science 2016; 353: 823–826.
31. Robbiani DF, Bozzacco L, Keeffe JR, Khouri R, Olsen PC and Gazumyan A: Recurrent potent human neutralizing antibodies to zika virus in brazil and mexico. Cell 2017; 169: 597–609.
32. Lok SM, Kostyuchenko V, Nybakken GE, Holdaway HA, Battisti AJ and Sukupolvi-Petty S: Binding of a neutralizing antibody to dengue virus alters the arrangement of surface glycoproteins. Nat Struct Mol Biol. 2008; 15: 312–317.

33. Tong JC and Ren EC: Immunoinformatics: Current trends and future directions. Drug Discov Today 2009; 14(13-14): 684-689.
34. Stadler MB and Stadler BM: Allergenicity prediction by protein sequence 2003; 17: 1141-1143.
35. Singh H, Raghava GPS: Propred I: prediction of HLA class I bindingsites. Bioinformatics 2003; 19: 1009–1014.
36. Gupta S: *In-silico* approach for predicting toxicity of peptides andproteins. Plo Sone 2013; 8: 73957.
37. Saha S and Raghava GPS: Prediction of Continuous B-cell Epitopes in an Antigen Using Recurrent Neural Network. Proteins 2006; 65(1): 40-48.
38. Doytchinova IA and Flower DR: Vaxi Jen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinformatics 2007; 8: 4.
39. Dhanda SK, Vita RHa B Grifoni A and Peters B, Sette A: Immunome Browser: a tool to aggregate and visualize complex and heterogeneous epitopes in reference proteins Bioinformatic 2018; 34(22): 3931-3933.
40. Shapovalov M, Vucetic S, Dunbrack RL and Shapovalov M: PLoSComput Biol 2019; 15(3): 1006844.
41. Kodama Y, Mashima J, Kosuge T and Ogasawara O: DDBJ update:the Genomic Expression Archive (GEA) for functional genomicsdata. Nucleic Acids Res 2019; 47: 69–73.
42. Sayers EW, Cavanaugh M, Clark K, Ostell J, Pruitt KD and Karsch-Mizrachi I: Gen Bank. Nucleic Acids Res 2019; 47: 94–99.
43. James R, Dominic JB, Xenia G, Michael AC, Paul Fk and Steven GEM: Nucleic Acids Research 2020; 48: 948–955.
44. Madeira F, Park YM, Lee J, Buso N, Gur T, Madhusoodanan N, Basutkar P, Tivey ARN, Potter SC and Finn RD: The EMBL-EBI search and sequence analysis tools APIs in 2019. Nucleic Acids Res 2019; 4: 636–641.

How to cite this article:
Dixit NK: Design *in-silico* multipathogenic vaccine of dengue and zika viruses using envelope protein. Int J Pharm Sci & Res 2022; 13(9): 3622-34. doi: 10.13040/IJPSR.0975-8232.13(9).3622-34.