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RESEARCH ARTICLE

Immuno-informatics-based Identification of Novel Potential B Cell and T Cell Epitopes to Fight Zika Virus Infections

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Abstract: ***Background*:** Globally, the recent outbreak of Zika virus (ZIKV) in Brazil, Asia Pacific, and other countries highlighted the unmet medical needs. Currently, there are neither effective vaccines nor therapeutics available to prevent or treat ZIKV infection.

***Objective*:** In this study, we aimed to design an epitope-based vaccine for ZIKV using an *in silico* approach to predict and analyze B- and T-cell epitopes.

***Methods*:** The prediction of the most antigenic epitopes has targeted the capsid and envelope proteins as well as non-structural proteins NS5 and NS3 using immune-informatics tools PROTPARAM, CFSSP, PSIPRED, and Vaxijen v2.0. B and T-cell epitopes were predicted using ABCpred, IEDB, TepiTool, and their toxicity was evaluated using ToxinPred. The 3-dimensional epitope structures were generated by PEP-FOLD. Energy minimization was performed using Swiss-Pdb Viewer, and molecular docking was conducted using PatchDock and FireDock server.

***Results*:** As a result, we predicted 307 epitopes of MHCI (major histocompatibility complex class I) and 102 epitopes of MHCII (major histocompatibility complex class II). Based on immunogenicity and antigenicity scores, we identified the four most antigenic MHC I epitopes: MVLAILAFLR (HLA-A*68:01), ETLHGTVTV (HLA-A*68:02), DENHPYRTW (HLA-B*44:02), QEGVFH TMW (HLA-B*44:03) and TASGRVIEEW (HLA-B*58:01), and MHC II epitopes: IIKKFKKDLAAMLRI (HLA-DRB3*02:02), ENSKMMLELDPPFGD (HLA-DRB3*01:01), HAET WFFDENHPYRT (HLA-DRB3*01:01), TDGVYRVMTRRLLGS (HLA-DRB1*11:01), and DGCW YGMEIRPRKEP (HLA-DRB5*01:01).

***Conclusion*:** This study provides novel potential B cell and T cell epitopes to fight against Zika virus infections and may prompt further development of vaccines against ZIKV and other emerging infectious diseases. However, further investigations for protective immune response by *in vitro* and *in vivo* studies to ratify immunogenicity, the safety of the predicted structure, and ultimately for the vaccine properties to prevent ZIKV infections are warranted.

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1. INTRODUCTION

Zika virus (ZIKV) is an arbovirus which was discovered in the late 1940s in a Rhesus monkey during a study of yellow fever transmission in forests in Uganda [1]. In 1952, the first human isolation of this virus occurred during an investigation in eastern Nigeria into the jaundice epidemic [2]. Belonging to the family *Flaviviridae*, this genus *Flavivirus* is transmitted mainly by mosquitoes of the genus Aedes [3]. Since the detection of the virus in Brazil in 2015, it has become a major public health challenge in the Americas. On February 1st, 2016, the World Health Organization (WHO) declared the major neurological complications of ZIKV, including the appearance of microcephaly and Guillan-Barré Syndrome, a global public health emergency [2]. In May 2017, the epidemic raged in 48 countries and territories of the Americas and Asia Pacific region [4, 5].

The ZIKV genome is located within a capsid of icosahedral symmetry and contains a single-strand positive direc-

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tion RNA molecule of 10,794 nucleotides composed of two non-coding regions (5' and 3' NCR) and an open reading frame (ORF) coding for a 3419 amino acid long polyprotein. The latter contains three structural proteins which are part of the viral particle, the capsid (C), pre-membrane (M) and envelope (E) proteins for which the E-DIII domain may be the best target for peptide vaccine development, as well as seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) that play a crucial role in RNA replication. NS5 protein (~103 kDa) is the largest and best-preserved viral protein that contributes to the regulation and multiplication of the virus. This NS5 protein has two distinct domains, a C-terminal domain with RNA-dependent RNA polymerase (RdRp) activity, which plays a vital role in RNA synthesis by catalyzing its replication, and an N-terminal domain with methyltransferase activity which is necessary for viral genome synthesis and capping. NS3 contains two enzymatic domains, an N-terminal serine protease activity of which NS2B is a cofactor [6], which leaves the viral protease a captivating target for antiviral discovery [7], and the C-terminal helicase, which directly participates in RNA replication. NS1 is a promising vaccine target that eliminates the risk of antibody-dependent enhancement (ADE) [8]. It plays a crucial role in viral replication and regulation of T cell responses to cell mediation, thus providing immune protection for ZIKV [9]. Other NS proteins are involved in virion assembly and maintaining the replication complex at the intracellular membrane level [6].

Currently, there are neither vaccines nor ZIKV-specific therapeutics available to prevent and treat infections, respectively [10]. Previous studies showed that capsid protein, envelop protein, NS5 RdRp, NS3 protease, and NS1 in mice, non-human primates, and in humans-infected with ZIKV, induce a strong antiviral CD4+ and CD8+ T cells immune response [8, 11, 12]. Hence, the objective of our study is to combine computer simulation knowledge (immuno-informatics), based on B- and T-cell epitopes, to design a new peptide vaccine to tackle ZIKV infections based on the whole genome.

2. MATERIALS AND METHODS

2.1. Data Collection

The structural protein (capsid and envelope protein) sequences, as well as the three non-structural proteins (NS5 RdRp, NS3 protease, and NS1), were extracted from the PDB database (https://www.rcsb.org/) [13] in FASTA format. In the absence of a protein crystal, the HLA (human leukocyte antigen) molecules of the MHC I and MHC II receptors were obtained by homology modeling. The coordinates of the atoms of the protein whose structure has been resolved by crystallography called "Template" have been generated from the Uniprot database (www.uniprot.org) [14]. The sequence was recorded in FASTA format and then used to reconstruct the three-dimensional structure of the proteins studied "Target."

The homology research, the calculation of the identity percentage, and the alignment were performed using SWISS-MODEL, a webserver dedicated to the homology modeling of protein structure [15].

2.2. Sequence Analysis by Physicochemical Characterization

The various physical and chemical parameters of the structural and non-structural protein sequences, such as molecular weight, theoretical pI, amino acid composition, extinction coefficient, were analyzed by PROTPARAM, a server available on https://web.expasy.org/protparam/ [16].

2.3. Secondary Structure Prediction

The secondary structures were predicted by CFSSP (http://www.biogem.org/tool/chou-fasman/), a server for predicting the secondary structure of proteins from the amino acid sequence, using the Chou & Fasman algorithm [17] and validated on PSIPRED [18].

2.4. Protein Antigenicity Prediction

Antigenicity of proteins and epitopes were estimated using VaxiJen v2.0 (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html), a reliable prediction server, with an accuracy of 70% to 89% [19].

2.5. B-Cell Epitopes Prediction

The prediction of linear epitopes of B cells was performed using ABCpred (https://webs.iiitd.edu.in/raghava/abcpred/index.html), based on an artificial neural network with a default specification of 65.93% [20]. Discontinuous epitopes were predicted using the ElliPro suite of Immune Epitope Database and Analysis Resource (IEDB) (http://tools.iedb.org/ellipro/) based on the three-dimensional structure of a protein antigen of a given sequence. This server is based on the Thornton method and uses two programs in parallel: MODELLER and Jmol to visualize the discontinuous epitopes of B cells in 3D [21].

2.6. T-cell Epitopes Prediction

The prediction of MHC-I epitopes was determined using the Immune Epitope Database and Analysis Resource (IEDB) **(**http://tools.iedb.org/mhci/), which compiles experimental data on antibody and T cell epitopes studied in humans and other species from various infectious diseases and autoimmunity [22]. The binding predictions of MHC II were made with the IEDB analysis resource, TepiTool (http://tools.iedb.org/tepitool/) [23], using the Consensus [24, 25] method which uses SMM_align, NN_align, the combinatorial library, Sturniolo, and NetMHCIIpan [26, 27] while specifying the species and human locus DR, DP, and DQ, as well as the alleles.

The immunogenicity of the predicted epitopes was calculated by the immunogenicity predictor of T cell class I pMHC (http://tools.iedb.org/immunogenicity/) using the properties of the amino acids and their position in the peptide [28].

2.7. Epitope Toxicity Evaluation

The prediction of the toxicity of the epitopes was performed using ToxinPred, a useful in silico method, developed to predict toxic or non-toxic peptides, available at the link: https://webs.iiitd.edu.in/raghava/toxinpred/index.html [29].

2.8. Energy Minimization

Energy minimization is an important step in finding a stable structure of protein sequences to prepare systems before applying other methods such as molecular docking. For this step, we used Swiss-PdbViewer, an engine allowing to perform an energy minimization, available on the following link: https://spdbv.vital-it.ch/refs.html [30].

2.9. Molecular Docking

Before starting the molecular docking study, the prediction of the three-dimensional structures of epitopes was performed using the PEP-FOLD online server available on: http://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD/, that aims to predict the 3D structures of peptides based on the amino acid sequence [31, 32]. Subsequently, PatchDock, a dedicated molecular docking server, was used to predict the Ligand-Receptor complex structure (https://bioinfo3d.cs.tau.ac.il/PatchDock/php.php) [33], and the type of complex used for docking is the protein-small ligand complex whose algorithm uses a parameter set optimized for small molecules. Finally, FireDock (http://bioinfo3d.cs.tau. ac.il/-FireDock/php.php) is an efficient method that allows the refinement of fast interaction in molecular docking [34, 35].

3. RESULTS

3.1. Sequence Analysis by Physico-Chemical Characterization

The capsid protein of ZIKV was composed of 104 amino acids, with a molecular weight of 11,861.61 Daltons, an extinction coefficient of 5500 M-1cm-1, an aliphatic index of 116.35, and a theoretical pI of 12.02. For the amino acid composition, Arg (R) and Leu (L), were the most common amino acids, with a percentage of 13.5% while Asp (D), His (H), Thr (T), and Trp (W) were the least common with a percentage of 1%. A hydropathy index of -0.119, and the instability index of 43.72 classified the protein as unstable.

The envelope protein of ZIKV consisted of 500 amino acids, with a molecular weight of 54,085.86 Daltons, an extinction coefficient of 72,140 M-1cm-1, an aliphatic index of 80.90, and a theoretical pI of 6.48. Also, the amino acid composition analysis revealed Gly (G) as the most frequent amino acid with a percentage of 11%, while Trp (W) is the least frequent amino acid with a percentage of 2%. A hydropathy index of -0.068, and last but not the least, the instability index of 21.76 indicated that the protein to be stable.

The non-structural protein NS5 RdRp was composed of 903 amino acids, with a molecular weight of 10,3015.47 Daltons, an extinction coefficient of 22,5710 M-1cm-1, an aliphatic index of 73.81, and a theoretical pI of 8.67. As far as amino acid composition is concerned, Gly (G) was the most frequent (9.2%), while Lys (L) was the less frequent (1.9%). Also, a hydropathy index of -0.541, and the instability index of 40.73 classified the protein as unstable.

NS3 protease consisted of 448 AA, with a molecular weight of 47,344.96 Daltons, an extinction coefficient of 87,110 $M\text{-}1cm^{-1}$, an aliphatic index of 78.26, and a theoretical pI of 5.01. Besides, the amino acid composition analysis showed Gly (G) with a high percentage of 16.5%, while His (H) and Phe (F) were the least frequent at 1.3%. The hydropathy index of -0.330 and the instability index of 30.68 implied that the protein was stable.

The NS1 consisted of 362 AA, with a molecular weight of 41,450.88 Daltons, an extinction coefficient of 89,920 $M\text{-}1cm^{-1}$, an aliphatic index of 62.49, and a theoretical pI of 5.85. Moreover, the amino acid composition analysis revealed Glu (E) as the most common amino acid, with a percentage of 12.7%, while Pyl (O) and Sec (U) were practically absent. The hydropathy index of -0.119 and the instability index of 55.18 indicated that this non-structural protein was unstable (Supplementary Table **1**).

3.2. Prediction of Secondary Structures

The analysis for the prediction of the secondary structure by CFSSP showed the percentages of alpha helixes, beta-sheets, and beta turns of each of the four proteins, as shown in Table **1**. On the other hand, PSIPRED validated the results with graphical output of proteins organized into coil regions interrupted by the alpha helixes in pink and the beta-sheets in yellow (Fig. **1**).

Table 1. Prediction of secondary structures of five ZIKV proteins using CFSSP

Protein	Alpha helix (H)	Beta-sheet (E)	Turns (T)
Capsid	77.8%	56.4%	71.6%
Envelope	48.9%	64.9%	60.7%
NS5 RdRp	9.3%	13.2%	13.2%
NS3 protease	58.3%	30.1%	14.8%
NS1	64.2%	24.5%	15.5%

3.3. Prediction of Protein Antigenicity

Using Vaxijen v2.0 server with a threshold of 0.4, the antigenicity of the two structural proteins, the envelope and capsid of the protein, were 0.5492 and 0.3856, respectively. The latter is probably considered as a non-antigen, unlike the envelope of protein that is probably considered as an antigen. Besides, the other three non-structural proteins, NS5 RdRp, NS3 protease, and NS1, were considered as antigens with antigenicity values of 0.4306, 0.4337 and 0.5983, respectively.

3.4. Prediction of B-Cell Epitopes

The prediction of B lymphocyte epitopes with the ABCpred server gave rise to 266 epitopes (16mer) in total for the five proteins (Supplementary Table 2), of which each

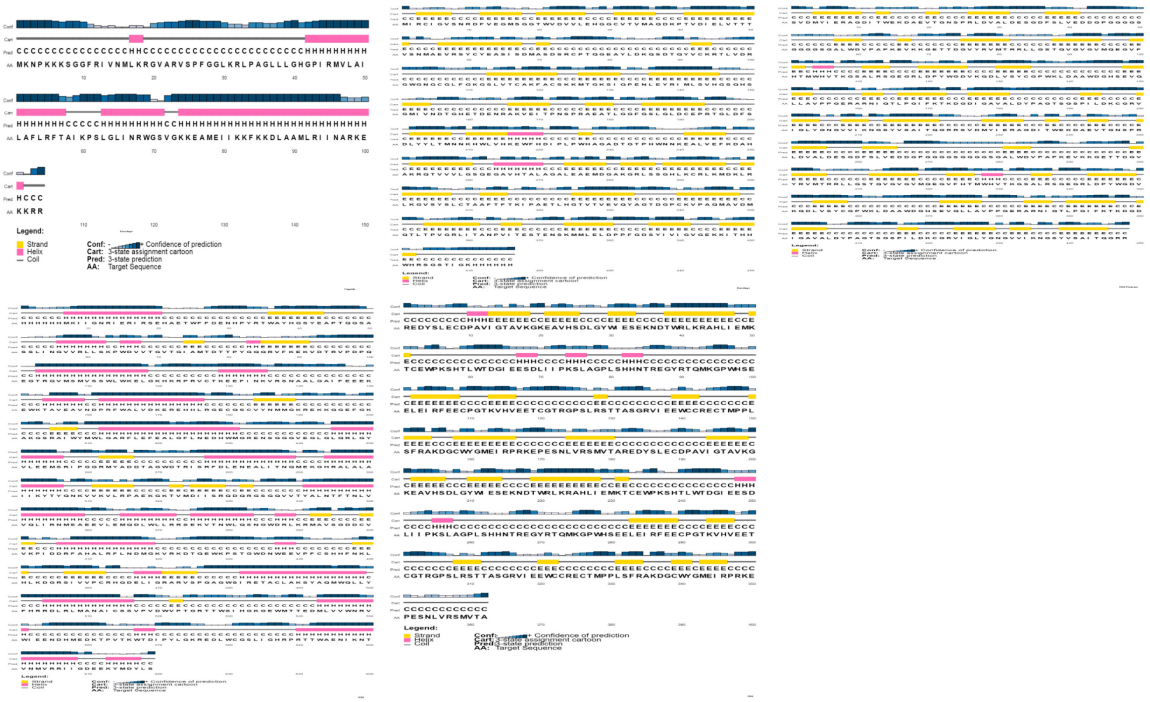

Fig. (1). Graphical output of the secondary structures of the ZIKV proteins using PSIPRED. *(A higher resolution / colour version of this figure is available in the electronic copy of the article)*.

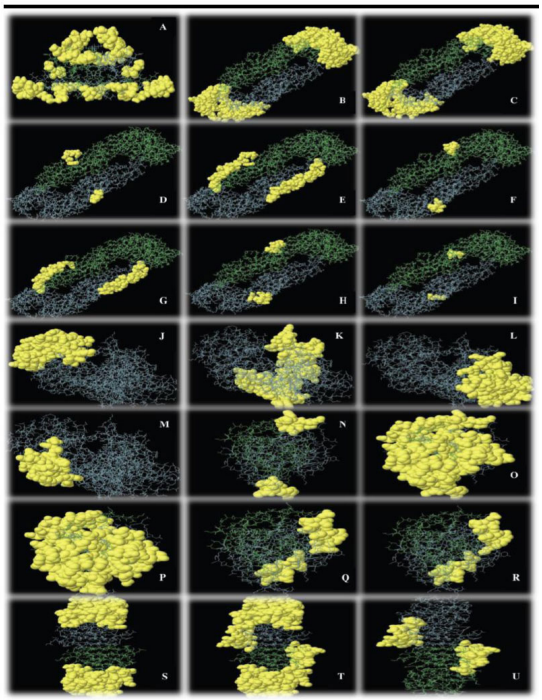

Fig. (2). 3D structural mapping of discontinuous B-cell epitopes of ZIKV proteins (in yellow). **(**A) Capsid protein. (B-I) Envelope protein. (J-M) Non-structural protein 5(NS5) RNA-dependent RNA-polymerase (RdRp). (N-S) NS3 Protease. (T-U) NS1 protein. *(A higher resolution / colour version of this figure is available in the electronic copy of the article)*.

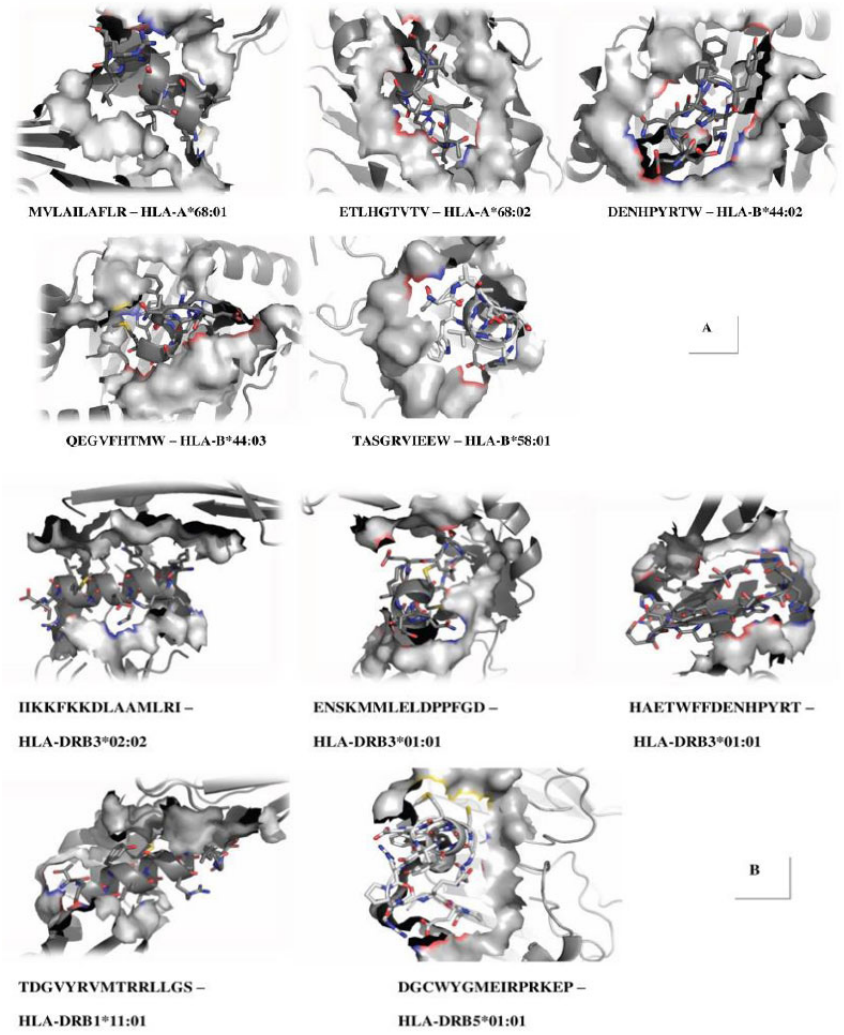

Fig. (3). Representations of the docked poses of the complexes. **(A)** Represents the MHC I alleles, and **(B)** represents the MHC II alleles docked with the most effective peptides. These peptides are color-highlighted according to each element (grey-carbon, redoxygen, blue-nitrogen), and the yellow lines represent hydrogen bridges. *(A higher resolution / colour version of this figure is available in the electronic copy of the article)*.

epitope had a score less than or equal to 0.95. The prediction of conformational epitopes was determined using ElliPro. These discontinuous epitopes have been defined according to the protrusion index values, and were grouped according to distance R (in Å). A supplementary table 3 shows the discontinuous B cell epitope residues of each protein with a score of ≥ 0.5. The structure of the discontinuous 3D epitopes was generated using the two programs MODELLER and Jmol by ElliPro (Fig. **2**). In addition, the antigenicity score of the epitopes was determined using the Vaxijen v2.0 server with a default threshold of 0.4 for each epitope. The data showed that the epitopes might be antigenic or not. Among the linear peptides, 154 antigenic epitopes were predicted with an antigenicity score higher than 0.4, as well as for the 17 discontinuous epitopes. Hence the most powerful continuous and discontinuous epitopes are listed in Table **2**.

3.5. Prediction of T Cell Epitopes

Using the IEDB server, we were able to predict the CMHI alleles of the viral proteins, which gave us a total of potent epitopes of 30, 62, 153, 40 and 24 for capsid protein, envelope protein, NS5 RdRp, NS3 protease, and NS1, respectively (Supplementary Tables 4, 5, 6, 7 and 8), each line of these tables corresponds to a prediction of the peptide bond. The columns contain the allele for which the prediction was made, the starting and ending position of the peptide, its length, the sequence of the peptide, and the percentile rank. The selection of predicted binders was made based on the percentile rank of ≤ 0.5% to cover most immune responses.

In the capsid protein, the most potent epitope is a 10mer MVLAILAFLR (46-55 aa), which had the lowest percentile

Table 2. Most effective continuous- and discontinuous ZIKV epitopes of B cells for all five proteins

Protein	Continuous Epitopes		Discontinuous Epitopes	
	Peptide	Score	Peptide	Score
Capsid	VLAILAFLRFTAIKPS	0.77	A:A35, A:G36, A:L38, A:L39, A:G40, A:H41, A:G42, A:A58, A:I59, A:K60, A:P61, A:L63, A:S71, A:V72, A:K75, A:K86, A:R93, A:E100, A:K101, A:K102, A:R103, A:R104, B:A35, B:G36, B:L38, B:L39, B:G40, B:H41, B:G42, B:A58, B:I59, B:K60, B:P61, B:L63, B:S71, B:V72, B:K75, B:E79, B:R93, B:E100, B:K101, B:K102, B:R103, B:R104	0.661
Envelope	TVSNMAEVRSYCYEAS	0.71	B:L196, B:D197, B:S199, B:D200	0.519
NS5 RdRp	GWDNWEEVPFCSHHFN	0.70	A:H719, A:L720, A:K721, A:D722, A:G723, A:R724, A:S725, A:F769, A:R772, A:T807, A:T808, A:E809, A:D810, A:L812, A:V813, A:N816, A:R817, A:I820, A:E821, A:E822, A:N823, A:D824, A:H825, A:M826, A:D828, A:K829, A:T830, A:P831, A:V832, A:T833, A:K834, A:W835, A:T836, A:D837, A:I838, A:P839, A:Y840	0.672
NS3 protease	RGXPDIDCHAINSEQE	0.72	B:G1055, B:A1057, B:R1059, B:S1060, B:G1061, B:E1062, B:G1063, B:R1064, B:L1065, B:D1066, B:P1067	0.647
NS1	HEMISTRYREMARKST	0.97	A:S176, A:L177, A:C179, A:D180, A:A182, A:V183, A:I184, A:D197, A:L198, A:G199, A:I218, A:E219, A:M220, A:K221, A:T222, A:C223, A:E224, A:W268, A:E272, B:G190	0.608

Table 3. Most effective epitopes for MHC-I alleles of ZIKV proteins

Protein	Allele	Peptide	Length	Immunogenicity score
Capsid	HLA-A*68:01	MVLAILAFLR	10	0.28372
Envelope	HLA-A*68:02	ETLHGTVTV	9	0.15601
NS5 RdRp	HLA-B*44:02	DENHPYRTW	9	0.08253
NS3 protease	HLA-B*44:03	QEGVFHTMW	9	0.12715
NS1	HLA-B*58:01	TASGRVIEEW	10	0.33928

Table 4. Most effective ZIKV epitopes for MHC-II alleles.

Protein	Allele	Peptide	Score
Capsid	HLA-DRB3*02 :02	IIKKFKKDLAAMLRI	0.01
Envelope	HLA-DRB3*01:01	ENSKMMLELDPPFGD	0.01
NS5 RdRp	HLA-DRB3*01:01	HAETWFFDENHPYRT	0.01
NS3 protease	HLA-DRB1*11 :01	TDGVYRVMTRRLLGS	0.01
NS1	HLA-DRB5*01:01	DGCWYGMEIRPRKEP	0.77

value of 0.12 for HLA-A*68:01 and an IC_{50} binding affinity threshold of 6.30 nM (Supplementary Table 4).

For the envelope protein, the most potent epitope was a 9mer epitope ETLHGTVTV (321-329 aa), which had the lowest percentile value of 0.1 and a binding affinity threshold of 6.60 nM for HLA-A*68:02 (Supplementary Table 5).

In the NS5 RdRp, the most potent epitopes were a 9mer MSMVSSWLW (107-115aa), 9mer DENHPYRTW (28-36aa), and 9mer QEWKPSTGW (426-434aa) with the lowest percentages of 0.06 for HLA-B*57:01, HLA-B* 44:02, and HLA-B* 44:03, respectively and a binding affinity threshold of 3.41, 12.53, and 22.15 nM, respectively (Supplementary Table 6).

For the NS3 protease, the most potent epitope was a 9mer QEGVFHTMW (96-104 aa) with the lowest value of 0.11 for the allele HLA-B*44:03 as well as an IC_{50} binding affinity threshold of 20.62 nM (Supplementary Table 7).

For the NS1 protein, the most important epitope was 10mer TASGRVIEEW (131-140 aa), with the lowest percentile value of 0.15 for the allele HLA-B*58:01 and an IC50 binding affinity threshold of 18.08 nM (Supplementary Table 8).

For the prediction of CMHII alleles of proteins by IEDB (Tepitool), data were generated by selecting peptides with a percentile rank of ≤1 by default for the five viral proteins (Supplementary Tables 9, 10, 11, 12, and 13).

3.6. Evaluation of Epitope Toxicity

To determine the toxicity of these peptides, the ToxinPred tool generated and assessed their various physicochemical and toxicity properties and specifically revealed that they were non-toxic.

3.7. Energy Minimization

The energy minimization was performed by Swiss-Pdb-Viewer, and we obtained potential energies, equal to -1609.464 Kcal/mol, -7500.717 Kcal/mol, -8512.906 Kcal/-mol, -2685.707 Kcal/mol, -3929,025 Kcal/mol for the cap-

sid, envelope, NS5 RdRp, NS3 protease, and NS1, respectively.

3.8. Molecular Docking Study

After obtaining the three-dimensional structures of the viral peptides, a molecular docking study was performed on the PatchDock server. The 20 best solutions were generated, each line representing the candidate complexes between the receptor and the ligand. The 10 best solutions were transferred to FireDock to be refined according to global energy, van der Waals (vdW), and Atomic Contact Energy (ACE) interactions. Hence the epitopes obtained from MHC I were: MVLAILAFLR, ETLHGTVTV, DENHPYRTW, QEGVFH TMW, and TASGRVIEEW (Table **3**). As well as the epitopes of MHC II are: IIKKFKKDLAAMLRI, ENSKMMLELDPPFGD, HAETWFFDENHPYRT, TDGVYRVMTRRLLGS, and DGCWYGMEIRPRKEP (Table **4**), which are illustrated in Fig. **3**, highlighting the active site.

4. DISCUSSION

To date, there is no specific treatment or vaccine for ZIKV infection. Prevention is based only on measures to control the mosquito transmission of the virus [10]. An immuno-informatics seems to be the most optimal and effective strategy for the development of a peptide vaccine. Previous studies have shown that the application of potential epitopes instead of the entire protein represents the complete antigenicity of the latter [36]. These epitopes can, therefore, stimulate a protective response by reporting the presence of an infection to the immune system, which allows them to be ideal components for the vaccine design. Hence in our study, we used an immuno-informatics process to predict the antigenicity of two structural proteins (capsid and envelope) and three non-structural proteins NS5 RdRp, NS3 protease, and NS1 as well as the identification of immunodominant epitopes of T and B lymphocytes.

Physico-chemical characterizations of proteins demonstrated that the most stable proteins were the envelope and NS3 protease with an instability index of 21.76 - 30.68, which indicated that they would more stable under *in vivo* conditions [37]. Furthermore, the other three proteins: capsid, NS5 RdRp, and NS1 successively had an instability index higher than 40: 43.72, 40.73, 55.18, suggesting their potential instability [37]. Whereas a high aliphatic index of 116.35 refers to the stability of the protein capsid at several temperatures, negative values of the hydropathy index, Grand Average of Hydropathy (GRAVY) show that proteins have strong interactions with H_2O and are hydrophilic [38].

The B-cell epitope, also known as an antigenic determinant or antibody epitope, is a molecule linked to a variable part of an antibody or B-cell receptor (BCR) [39, 40]. The prediction of these epitopes is a crucial step in the design of a peptide vaccine and immunodiagnostic tests [37]. There are two types of B cell epitopes, conformational or discontinuous epitopes. Conformational epitopes are defined as a set of amino acids from different parts of a polypeptide chain joined together by folding the protein whose longer distances allow the prediction of discontinuous epitopes covering large areas, while continuous or linear epitopes are composed of a single segment of a polypeptide chain (short peptide fragments) [21]. Moreover, the identification of antigenic epitopes and cross-reactive epitopes has a crucial role in vaccine and immunodiagnostic tool developments [11]. In this study, continuous epitopes B cell prediction revealed that epitope GGTWVDVVLEHGGCVT (452-467 aa) has a good antigenicity score with potential diagnostic use. In line with our data, Amrun et al., identified that peptide encompassing from amino acid residues 453 to 470 on E protein was the best performing ZIKV specific epitope that is able to distinguish between ZIKA-infected patients from other unknown infections [41].

T cell epitopes will be the most considered in our study since they are directly involved in vaccine development, and have been used in the majority of experimental studies. In the prevention of ZIKV infection, the prediction of T-epitopes (CD4+ and CD8+) played a critical role to have an effective vaccine [37]. In our results, we first had epitopes of MHC I with a lower percentile percentage indicating a high binding affinity and an IC_{50} binding affinity threshold of less than 50 nM, which classified the peptides to have a high affinity between MHC and the isolated peptide [42]. To further reduce the number of these candidates to be tested, we used the immunogenicity tool that predicts the relative ability of a peptide/MHC complex to trigger an immune response [42]. A positive score indicates a high probability of induction of an immune response by the peptide complex/MHC (T cell recognition), while a negative score indicates a less likely recognition [28]. For MHC II peptides, those with the lowest percentile score were used.

Peptides have several advantages, including low manufacturing costs and high biological activity that would play a crucial role in vaccine development [29]. Moreover, our data showed that the designed peptides were found to be non-toxic, allowing us to move directly to energy minimization. Among the factors that verify whether minimization has been successfully performed is the potential energy (Epot) that should be negative to have stability for protein structures [43], and which was the case in our study for the five proteins that are ready to be used for molecular docking studies.

Molecular docking plays a crucial role in the design of a peptide vaccine by studying the interaction between the receptor and its ligand. Besides, global energy and electrostatic interactions, resulting in the contribution of van der Waals forces to global binding energy, play an important role in binding affinity [44]. After obtaining the 20 solutions using PatchDock and moving to the second FireDock server, we were able to generate the 10 best-refined solutions based on global energy. The lower the global energy, the higher the binding affinity of the complex, which implies a more effective epitopic peptide [45]. Hence the most effective epitopes of MHC I: MVLAILAFLR (HLA-A*68:01) at position 46-55 of the capsid protein, of ETLHGTVTV (HLA-A*68:02) at position 321-329 of the envelope protein located much more precisely in domain III, which have al-

ready been mentioned as a better target for neutralization antibodies to develop a peptide vaccine [46]. Previous studies supported that several potent neutralizing monoclonal antibodies against the ZIKV target the E protein domain III [47, 48]. Notably, very highly similar epitopes identified in our study were previously identified as common epitopes (TPNSPRAEA – TPHWNNKEAL – YLDKQSDTQY - GLDFSDLYY - FSDLYYLTM – ELDPPFGDSY) and mostly are limited to HLA-A*0101 [49]. In line with our result, Elong Ngono *et al.,* identified putative epitopes in the E, NS1, NS3, and NS5 proteins able to induce a positive T cell response [50].

Regarding the NS5 RdRp, we found DENHPYRTW (HLA-B*44:02) at position 28-36 epitope, which also proved to be a better choice of target for drug discovery previously [51, 52]. Moreover, we found that the epitopes (APTQGSASSL, RPRVCTKEEF, RPRTTWAENI, and YAQMWQLLY) were mostly limited to HLA-A*0702 and major immunodominant epitopes [12, 50].

We then had the epitope QEGVFHTMW (HLA-B*44:03) at position 96-104 of the NS3 protease, and TASGRVIEEW (HLA-B*58:01) at position 131-140 of the NS1. The global energy values are -55.98, -58.27, -28.94, -47.72 and -9.82 for the most effective epitopes in the capsid protein, envelop protein, NS5 RdRp, NS3 protease, and NS1, respectively, and had a higher binding affinity for the receptors.

Similarly, MHC II peptides: IIKKFKKDLAAMLRI (HLA-DRB3*02:02) at position 80-94 of the structural protein: the capsid, ENSKMMLELDPPFGD (HLA-DRB3*01:01) at position 371-385 of the structural protein: the envelope, are still in domain III which confirms that our peptides can be the most effective in *in vitro* tests. HAETWFFDENHPYRT (HLA-DRB3*01:01) at position 21-35 of the NS5, TDGVYRVMTRRLLGS (HLA-DRB1*11:01) at position 73-87 of the NS3 protease, and DGCWYGMEIRPRKEP (HLA-DRB5*01:01) at position 156-170 of the NS1 with global energy of -38.37, -51.64, -81.64, -56.02 and -13.03 were considered effective epitopes with a higher binding affinity.

Previous works used the same approach to develop a peptide vaccine [53], using ProPred-I based on the antigenicity and immunogenicity score; the researchers suggested T cell epitopes (MHC I): QTLTPVGRL – RGGGTGETL – AAIEGEFKL – KGPWHSEEL; as having the potential to be vaccine candidates. Additionally, other T cell epitopes have shown a strong affinity to interact with MHC II alleles: MLRIINARKE – LGGFGSLGLLRLKGVSYS – LLYFHRRDLRLMANAICSS – LNQMSALEF – IKSVSTTSQ – VRSNAALGA - WYGMEIRPR. By comparing our results with Dar *et al.,* it turned out that there is no similarity, but we noticed that the MHC class II T-cell MLRIINARKE epitope sequence for protein capsid, present in our results, was included in two epitopes LAAMLRIINARKEKK – KDLAAMLRIINARKE related to alleles: HLA-DRB1*08:02 – HLA-DRB5*01:01 respectively with a score of 0.10 and 0.30. We also noticed that their WYGMEIRPR epitope for NS1 protein is included in our epitope DGCWYGMEIRPRKEP obtained for the CMH II allele [53]. Furthermore, recently published data reported that only the MVLAILAFLR epitope of the protein capsid is consistent with our study, so our epitope is linked to 3 of the 30 HLA class I alleles (HLA-A*68:01 – HLA-A*33:01 – HLA-A*31:01) [54]. With regard to the NS5 RdRp, we found that the epitope YMWLGARFL (HLA-A*02:01) was present in our results with a percentile rank of 0.4 and an IC_{50} of 15.74 nM, but we had to choose an epitope with a percentile rank below 0.06 which indicates a high binding affinity and an IC_{50} binding affinity threshold of less than 50 nM which indicates a high affinity between the peptide and the corresponding MHC. Regarding MHC II, we were able to distinguish that the sequence of their epitope found in FKKDLAAML (HLA-DRB1*09:01) for the capsid protein is included in our predicted epitope IIKKFKKDLAAMLRI (HLA-DRB3*02:02); the latter was chosen consistently in our study because of its percentile rank of 0.01 which allows a strong affinity.

CONCLUSION

In conclusion, using immunoinformatics-driven genome-wide screening of vaccine targets of ZIKV, we provide a short list of novel potential B cell and T cell epitopes. In addition, our results suggest that the predicted epitopes can stably interact with frequent HLA-I and HLA-II alleles. However, these data require further *in vitro* and *in vivo* studies along with this *in silico* study to corroborate the antigenicity of the epitopes for their efficient use as vaccines against ZIKV.

LIST OF ABBREVIATIONS

ACE = Atomic Contact Energy

BCR = B-cell receptor

GRAVY = Grand Average of Hydropathy

HLA = Human Leukocyte Antigen

IEDB = Immune Epitope Database and Analysis Resource

MHC I = Major Histocompatibility Complex class I

MHC II = Major Histocompatibility Complex class II

ORF = Open Reading Frame

Epot = Energy Potential

RdRp = RNA-dependent RNA polymerase

vdW = van Der Waals

WHO = World Health Organization

ZIKV = Zika virus

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

All authors have contributed significantly to this study and approved the content of the manuscript. We are particularly grateful to Fadila Guessous for pertinent English revision of the manuscript.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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None.

CONFLICT OF INTERESTS

The authors have no conflicts of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

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