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RESEARCH ARTICLE

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Epitope-based immunoinformatics approach on RNA-dependent RNA polymerase (RdRp) protein complex of Nipah virus (NiV)

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Abstract

Persistent outbreaks of Nipah virus (NiV) with severe case fatality throw a major challenge on researchers to develop a drug or vaccine to combat the disease. With little knowledge of its molecular mechanisms, we utilized the proteome data of NiV to evaluate the potency of three major proteins (phosphoprotein, polymerase, and nucleocapsid protein) in the RNA-dependent RNA polymerase complex to count as a possible candidate for epitope-based vaccine design. Profound computational analysis was used on the above proteins individually to explore the T-cell immune properties like antigenicity, immunogenicity, binding to major histocompatibility complex class I and class II alleles, conservancy, toxicity, and population coverage. Based on these predictions the peptide 'ELRSELIGY' of phosphoprotein and 'YPLLWSFAM' of nulceocapsid protein were identified as the best-predicted T-cell epitopes and molecular docking with human leukocyte antigen-C (HLA-C*12:03) molecule was effectuated followed by validation with molecular dynamics simulation. The B-cell epitope predictions suggest that the sequence positions 421 to 471 in phosphoprotein, 606 to 640 in polymerase and 496 to 517 in nucleocapsid protein are the best-predicted regions for B-cell immune response. However, the further experimental circumstance is required to test and validate the efficacy of the subunit peptide for potential candidacy against NiV.

KEYWORDS

epitope-based vaccine design, molecular docking, molecular dynamics, Nipah virus, nucleocapsid protein, phosphoprotein and polymerase

1 | INTRODUCTION

Time and again, Nipah virus (NiV) has proved itself as an emerging zoonotic threat to mankind. It was first reported in Malaysia and Singapore in the year 1998.[1] From then cases were recorded in different parts of Southeast Asia like Bangladesh, India, Malaysia, and Singapore.[2] A very recent outbreak was reported in May 2018 in the Indian state of Kerala (www.medicinenet.com/). According to World Health Organization (WHO),[3] the mortality rate of NiV virus is 100% (http://www.searo.who.int/entity/emerging_diseases/links/nipah_virus/en/). NiV, an RNA virus belongs to the hierarchical classification of the Paramyxoviridae family and genus Henipavirus which includes both Hendra

Abbreviations: ACC, autocross-covariance; HLA, human leukocyte antigen; IEDB, Immune Epitope Database; MHC, major histocompatibility complex; MSA, multiple sequence alignment; NiV, Nipah virus; RdRp, RNA-dependent RNA polymerase; RNA, ribo nucleic acid; SMM, stabilized matrix method; TAP, transporter associated with antigen processing; WHO, World Health Organization.

virus and Nipah virus. Nipah viral infection is characterized by encephalitis and respiratory illness with the symptoms manifesting itself in 2 weeks and can even cause coma.[4,5] Pteropus fruit bats are the natural reservoirs of NiV whereas the other hosts include foxes, horses, cats, and pigs.[6] Transmission to humans is through contaminated fruits, body fluids, secretions, and direct contact with infected humans[7] targeting the endothelial cells[8] with the production of cytokines that mediate inflammation eventually progressing to an acute respiratory distress syndrome[9] and encephalitis.[10] NiV is a highly virulent pathogen which is listed under Biosafety level 4.[11] The structural complexity and molecular mechanisms of pathogenicity are less explored and there is no effective treatment available. Ribavirin, an Food and Drug Administration (FDA) approved the antiviral drug for some RNA viruses[12,13] was not so effective in Nipah outbreaks.[14,15] Though NiV outbreaks are confined to the Southeast Asian countries it possesses a serious threat to the entire world as its host reservoir is widely distributed creating an urge for the development of efficacious vaccine.[4,16] Advancement in computational approaches in this post-genomic era has made epitope-based vaccine design as an imperative strategy to combat viral infections.[17,18] Recent reports suggest that this approach of vaccine development using epitope is highly potent against various pathogens.[19-21] Both T-cell and B-cell epitope predicting tools are available to assist the laborious conventional vaccine design. In B-cell epitope prediction any antigenic region that is solvent exposed is analyzed for evoking antibody production and it can be either linear or conformational to effectuate humoral immunity.[22] Whereas T cells epitope prediction looks for immunogenic antigen sequence which kindles cytotoxic T cells and T helper cells that mediates cell-mediated immunity.[23] Antigen processing, recognition by T-cell receptor, and major histocompatibility complex (MHC) binding are the key features for predicting T-cell epitopes.[24] The human immune system consists of a diverse range of polymorphic human leukocyte antigen (HLA) alleles and so these epitope peptides should recognize multiple HLA alleles. A potential epitope should bind to both class I and class II HLA alleles and have a substantial population coverage with no toxicity.[25,26]

The single-stranded RNA genome of NiV is 18.2 kb in length and codes for six proteins that includes 3′-nucleocapsid (N), phosphoprotein (P), matrix protein (M), fusion protein (F), glycoprotein (G), and polymerase (L)-5′.[27] With regard to Nipah virus, earlier studies have worked on epitope predictions in glycoprotein and a fusion protein of NiV.[4,20,28] Glycoprotein mediates host cell attachment and viral entry is mediated by F protein.[29] This study aims at deciphering the potentiality of three important structural proteins of Nipah virus—polymerase (L), nucleocapsid (N), and phosphoprotein (P) involved in viral replication and processing. These three proteins form the RNA-dependent RNA polymerase (RdRp) complex.[30] The RNA polymerase is meant to transcribe the Nipah viral messenger RNA (mRNA).[30] The nucleocapsid protein encapsidates the transcribed RNA and acts as a regulatory protein for transcription.[31] Phosphoprotein has multiple subunits and ties up the polymerase to nucleocapsid protein. Also it acts as a processivity factor to enhance the activity of polymerase.[32] Phosphoprotein interacts with nucleocapsid protein and polymerase protein separately before the RdRp complex is formed and these interactions are regulatory in function.[33] Since it is evident that the RdRp complex is obligatory for viral transcription this study aims to look for the efficacy of these three proteins in the complex—polymerase, phosphoprotein, and nucleocapsid protein to act as likely candidates for epitope-based vaccine design. The predicted epitopes can aid in further experimental studies as the products developed through immunoinformatics methods have gained seemingly increasing importance due to the advantage of being more safer and less expensive for screening.[34]

2 | METHODS

2.1 | Protein sequence retrieval and evolutionary analysis

The available sequences of three key proteins in NiV replication (phosphoprotein, polymerase, and nucleocapsid protein) were retrieved from UniProtKB database in FASTA format. These sequences that get lodged into the database are obtained from different regions of the world where NiV outbreaks have been reported.[4] Thirty-one sequences (7 for phosphoprotein, 7 for polymerase, and 17 for nucleocapsid protein) obtained were subjected to multiple sequence alignment (MSA) using MEGA-X software MEGA-X software, Pennsylvania State University, Tempe, Arizona, USA.[35] to compare sequence homology and examine the ancestral relationship.[36] The parameters were set to default with 1000 bootstrap value. A phylogenetic tree was also constructed using the same software with the maximum likelihood method.

2.2 | Prediction of antigenicity

Protein sequences were subjected to VaxiJen v2.0 (OMIC tools, Petit-Quevilly, France) to predict antigenicity.[19] Using autocross-covariance method[37] with the target organism as a virus, each of the sequences for all three proteins was individually analyzed. For phosphoprotein and nucleocapsid protein, the default threshold for viruses of 0.5 was used,[38] whereas the cutoff for polymerase protein was set to 0.4 as no antigenic sequence was obtained for the default threshold.[39]

From the results obtained, the probable antigenic sequence in each of the three proteins was subjected to the next step.

2.3 | Prediction of T-cell epitopes

NetCTL-1.2 server (DTU bioinformatics, Technical University of Denmark Kemitorvet, Building 208, Denmark) was used to anticipate cytotoxic T lymphocyte epitopes for 12 MHC class I supertypes (A1, A2, A3, A24, A26, B7, B8, B27, B39, B44, B58, and B62). The score for proteasome cleavage and transporter associated with antigen processing (TAP) transport efficiency involved in the course of antigen processing was calculated using simulated neural networks.[40] The peptide length was taken as 9 with the threshold set to be >0.5 providing a sensitivity of 0.89 and specificity of 0.940. The first five epitopes with a higher combined score in each protein were considered for further analysis.

2.4 | Binding to the MHC alleles

Using Immune Epitope Database (IEBD, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland) MHC I prediction tool, the HLA alleles of MHC class I bound by the five best epitopes from the previous results were identified.[41] Stabilized matrix method (SMM) was considered to manipulate bound data and provide quantitative output.[42,43] The IC_{50} value was set to $\leq$500 nM that determines the efficiency of the particular peptide to interfere with cellular activity. Fifteen epitope sequences, five from each protein were used against all the available HLA alleles of MHC class I. A vital characteristic of an efficient epitope is to bind to both MHC class I and II alleles.[20] Hence, the best epitope after being subjected to all the analysis was studied for class II MHC binding in the loci of HLA-DQ, DP, and DR region with the SMM-align method, with the same IC_{50} cutoff value.[44]

2.5 | Conservancy analysis

The rate of evolution and variability was calculated with the IEBD epitope conservancy analysis tool. Epitopes with highly conserved sequences exhibit immunity against a broad spectrum of isolated strains and can act as a good target for vaccine designing.[45] Following user-defined criteria, the percentage conservancy of the epitopes was identified with the upper limit for identity set to 100%.

2.6 | Toxicity, allergenicity, and immunogenicity prediction

One key aspect of epitope-based vaccine designing is that the particular epitope should elicit an immune response only against the target virus leaving the host cells and tissues unaffected.[46] Toxicity check was performed with ToxinPred (Bioinformatics Centre, Institute of Microbial Technology, Chandigarh, INDIA), an in silico tool anticipating toxicity based on physiochemical properties.[47] To verify that the vaccine developed is nonallergic and do not base a vigorous immune reaction, the epitopes were examined with an online server AllerTop 2.0 (Department of Chemistry, Medical University of Sofia, Bulgaria)[48,49] utilizing the autocross-covariance (ACC) method. While antigenicity refers to the ability of the antigen to bind to antibodies, immunogenicity explains if a given antigen can successfully provoke the immune system as not all antigens are good immunogens.[50] Epitopes with a positive score from IEDB immunogenicity prediction tool were considered as potent immunogens.[51]

2.7 | Population coverage

Though NiV outbreaks are confined to the Southeast Asian regions, there is a universal threat of Nipah viral infection because of the distribution of its host reservoir.[4] Thus, it is inevitable to evaluate the rate at which the epitope covers the entire population. Based on the results on all the above analysis the best epitope was identified in each of the proteins (three in total) and was subjected to IEDB population coverage tool[52] with specific references to countries like Malaysia, India, and Singapore where outbreaks have been reported and the cumulative percentage of the coverage was obtained.

2.8 | Molecular docking and dynamics simulation

Docking was carried out to comprehend the binding affinity of the epitope with the MHC I molecule. Since most of the epitopes were seen to bind to HLA-C*12:03 molecule, this allele was selected as the target protein for docking.[19] The best epitope filtered through the above process from phosphoprotein and nucleocapsid protein was set as ligands to be docked against the HLA molecule. Five three-dimension (3D) structures for these three epitopes were obtained from the online server PEP-FOLD 2.0 (RPBS French Institute of Bioinformatics, Orsay University Campus, France) in PDB format.[53,54] The crystal structure of HLA-C*12:03 was retrieved from RCSB Protein Data Bank (PDB ID: 1EFX).[55,56] The chains C, D, and E were removed from the molecule.[57] The residues of the HLA-C*12:03 active site were predicted using the CASTp 3.0 server (Wei Tian and Jieling Zhao, University of Illinois at Chicago, IL).[58] All the water molecules were removed before docking using PyMOL (Schrödinger, NY) [L[59]] and the energy was minimized using Swiss PDB viewer (Swiss Institute of bioinformatics, QuartierSorge - BatimentGenopode1015

LaUSAnne/Switzerland).[60] Using AutoDock Vina (The Scripps Research Institute (TSRI) San Diego, CA)[61] the protein-peptide docking was achieved with default settings having a grid size of *x*-axis—36.679, *y*-axis—7.464, *z*-axis—53.10 specified using AutoDock 4.2 (The Scripps Research Institute (TSRI), San Diego, CA).[62] The energy range was set to 8 with 10 outputs. To validate the docking results, we have repeated it three times with the default parameters.[63] Docking was also performed using PatchDock (Dina Duhovny, School of Computer Science, Tel Aviv University, Israel),[64] a web-based algorithm for protein-peptide docking and CABS-dock (Laboratory of Theory of Biopolymers, University of Warsaw, Warsaw, Poland)[65] an interface to model protein-peptide interactions.

Molecular dynamics (MD) simulation was performed using GROMACS 4.5.6 (University of Groningen, KTH Royal Institute of Technology, Uppsala University, Sweden) to analyze the changes in structural confirmation of the protein when bound to the epitope.[66] The obtained docked complex was converted into gromos file format and a cubic box of 1.0 cm was set using the editconf command. This was followed by protein solvation in genbox with water molecules by utilizing simple point charge (spc) water mode and energy minimization were done using GROMOS 43A1 force field. Ensuring chlorine incorporated to neutralize the system. Using the isothermal-isochoric ensemble to equilibrate the temperature and pressure (NVT and NPT). Finally, the MD simulation was run up to 100 ns and the trajectories used for further analysis.

2.9 | B-cell epitope prediction

A good epitope candidate should be capable of inducing both cell-mediated and humoral immunity for effective pathogenic elimination.[67] Accessibility and antigenicity are the key characteristics of B-cell epitopes. Based on amino acid propensity scales B-cell epitopes were identified using online tools from IEBD resource in a sequential manner for all three proteins individually.[68] The protein sequence with the highest VaxiJen 2.0 score was further taken for the predictions.

Antigenicity was determined using the Kolaskar and Tongaonkar antigenicity prediction (Immune Epitope

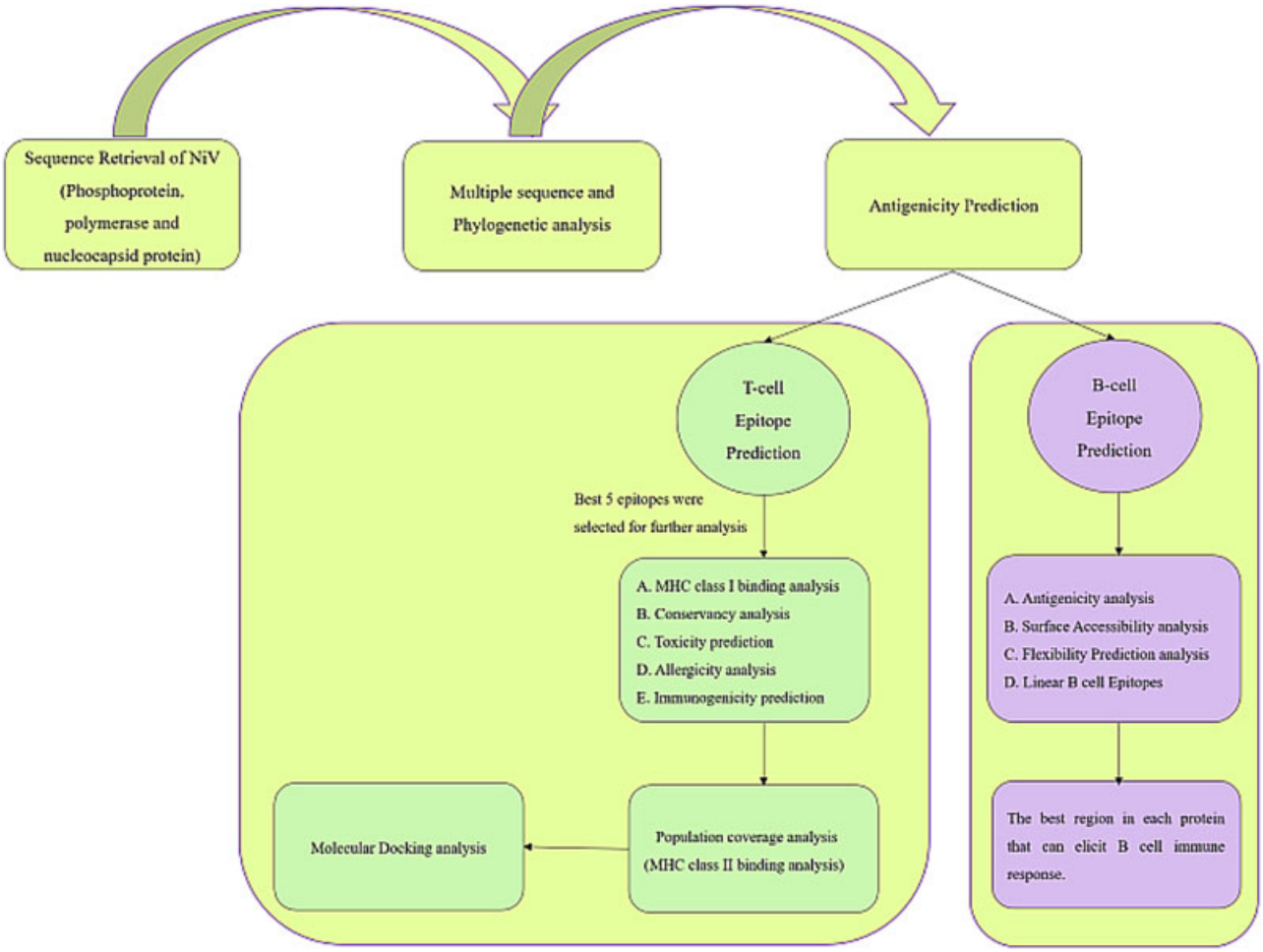

FIGURE 1 The overall methodology of the work is depicted. MHC, major histocompatibility complex; NiV, Nipah virus

Database (IEDB) National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MA)[69] and the threshold value was set to 1.00. The surface accessibility was speculated with Emini surface accessibility prediction (Immune Epitope Database (IEDB) National Institute of Allergy and Infectious Diseases, NIH, Bethesda,MA).[70] The Chou and Fasman scale method (Immune Epitope Database (IEDB) National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MA)[71] was implied for β-turn prediction whereas flexibility was studied using Karplus and Schulz flexibility scale (Immune Epitope Database (IEDB) National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MA).[72] To assess if the epitopes are hydrophilic, Parker Hydrophilicity prediction (Immune Epitope Database (IEDB) National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MA)[73] with default threshold level was implied. To locate the linear B-cell epitopes precisely BepiPred linear epitope prediction (Immune Epitope Database (IEDB) National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MA) was used involving Hidden Markov statistical model (Immune Epitope Database (IEDB) National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MA) and propensity scale (Immune Epitope Database (IEDB) National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MA) method.[74]

3 | RESULTS

The overall methodology of the work is depicted in Figure 1.

3.1 | Evolutionary analysis

Multiple sequence alignment revealed that the sequences obtained from multiple strains were highly conserved with more than 90% in phosphoprotein, 98% in nulceocapsid protein, and 98% in polymerase protein. The evolutionary divergence of sequences from each of the three protein is explained in Figure 2A-C.

3.2 | Antigenicity prediction

Though all the sequences had a VaxiJen v2.0 score above the cutoff level, the out turn indicated that the sequence with UniprotKB ID: E2II04 of phosphoprotein had the highest score of 0.5905 and in nucleocapsid protein the sequence with UniprotKB ID: H6V858 had the highest score of 0.5875 with the default cutoff 0.5. In the case of polymerase, where the cutoff used was 0.4, the protein sequence with UniprotKB ID: Q4VCP4 with the highest score 0.4773 was selected. These three sequences are taken to be potential antigens and were used for further analysis. The UniprotKB accession ID and antigenicity scores for all the sequences are given in Supporting Information Table 1.

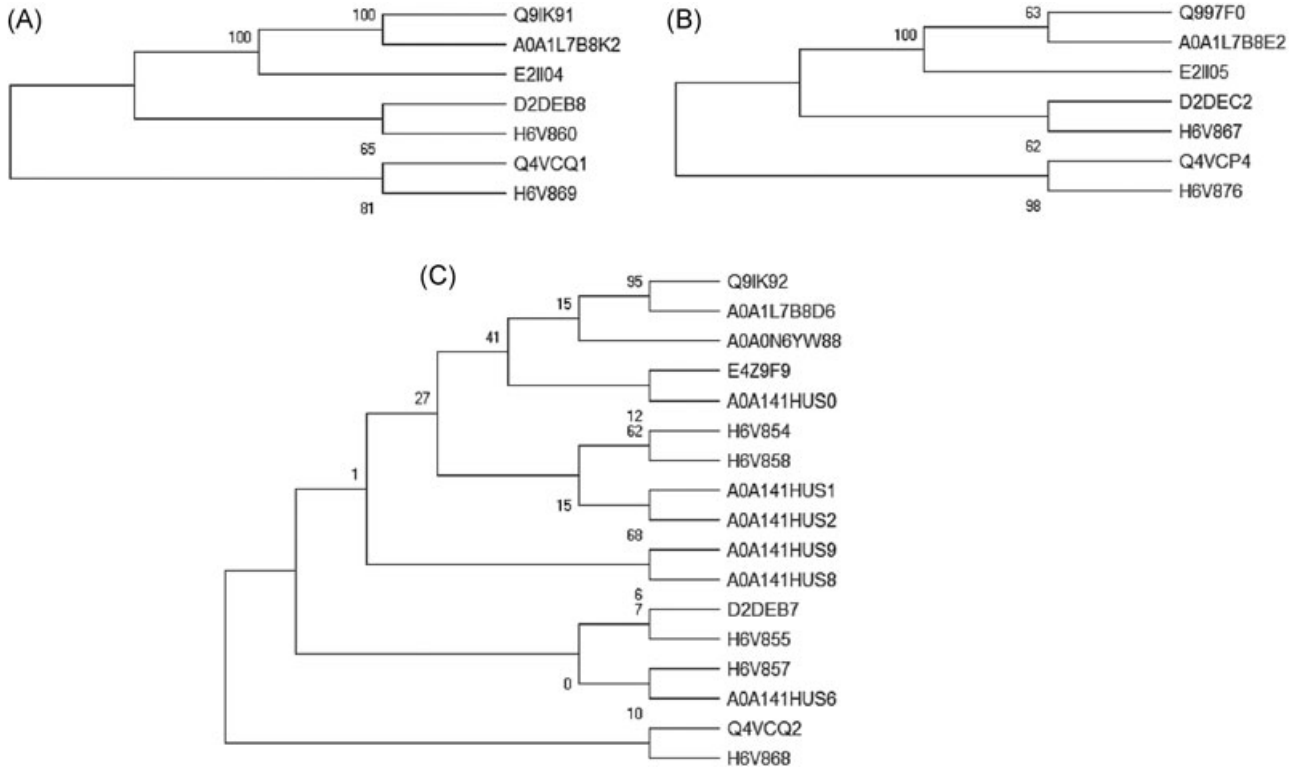

FIGURE 2 Phylogenetic analysis for protein sequences of different strains of Nipah virus isolated so far. The figure represents evolutionary divergence of A, phosphoprotein; B, polymerase, and C, nucleocapsid protein

TABLE 1 First five epitopes identified using NetCTL-1.2 server with their antigen processing and antigenicity scores

S. no	Peptide sequence	Start position	Clevage score	TAP score	COMB score	VaxiJen 2.0 score
			Phosphoprotein	SEQ ID	E2II04	
1	VSDAKMLSY	151	0.6191	2.899	3.6858	0.6748
2	PSDDFSNTF	480	0.9467	1.955	2.0706	0.0045
3	REDLILPEL	634	0.9715	0.935	1.9367	0.2085
4	MPKSRGIPI	396	0.9475	0.13	1.8906	2.0037
5	ELRSELIGY	676	0.7875	2.921	1.8682	0.9653
			Polymerase	SEQ ID	Q4VCP4	
1	ETDDYNGIY	1412	0.9526	2.571	3.7225	0.3789
2	NTSKSFLDY	673	0.9649	2.99	3.5486	0.2967
3	EVDNNHLIY	1450	0.9747	2.693	3.3352	0.3101
4	LTPEMVLMY	230	0.9575	2.84	3.2545	0.1542
5	RPRGRHTMV	1562	0.9746	0.435	1.8812	0.9197
			Nucleocapsid protein	SEQ ID	H6V858	
1	EIISDIGNY	250	0.7551	2.965	2.602	−0.2042
2	YPLLWSFAM	327	0.945	−0.032	2.1105	0.9259
3	TIKSLMLLY	293	0.9032	3.171	2.0422	0.6166
4	RRWAKYVQQ	192	0.4452	0.527	1.8455	0.0357
5	SRLAAKAAK	481	0.8902	0.874	1.7996	0.9631

3.3 | T-cell epitope prediction

The combinatorial score explaining the protein cleavage and TAP score was the criteria for identifying the best five epitopes from the NetCTL server. In addition, these epitopes were screened individually using VaxiJen v2.0 for antigenicity in which few epitopes had scores below the set cutoff. The list of best five epitopes with relevant scores of cleavage, TAP, combinatory, and antigenicity is given in Table 1.

3.4 | MHC I binding prediction

Because of the diversity in MHC alleles, it is necessary to evaluate that these epitopes can bind to multiple HLA alleles to cover a broad spectrum. The epitopes and its respective HLA alleles are depicted in Table 2. Among all the epitopes, ELRSELIGY in phosphoprotein, RPRGRHTMV in polymerase, and YPLLWSFAM in nucleocapsid protein were found to bind with the maximum number of HLA alleles. The antigen processing scores are depicted in Supporting Information Table 2.

3.5 | Conservancy, toxicity, allergenicity, and immunogenicity prediction

Out of five epitopes in phosphoprotein, three epitopes had 100% conservancy and two epitopes had 43% conservancy. A 100% conservancy was observed in both polymerase and nucleocapsid epitopes except for one epitope, which is 94% conserved in nucleocapsid protein. The results obtained from ToxinPred to affirm nontoxic immunization rendered all the 15 epitopes to be nontoxic and can be safely used. On the other hand, allergenicity results revealed that four epitopes of phosphoprotein, three epitopes of polymerase, and two epitopes of nucleocapsid protein to be nonallergic and the rest were allergic which could induce T helper cells and IgE antibodies to over sensitize the host cells.[75] Three epitopes from phosphoprotein, three epitopes from polymerase, and two epitopes from nucleocapsid protein were immunogenic in the range of 0.01 to 0.17. From all the above analysis, the best epitopes in each protein were narrowed down to ELRSELIGY in phosphoprotein, RPRGRHTMV in polymerase, and YPLLWSFAM in nucleocapsid protein.

The scores for conservancy, toxicity, allergenicity, and immunogenicity are listed in Table 3.

3.6 | Population coverage analysis

The cumulative percentage of population coverage uncovered that the epitope ELRSELIGY had the highest coverage of 95.39% in Europe and the least in South Africa with a percentage of 1.46% and in India, the coverage could be 76.96%. The epitope RPRGRHTMV of polymerase had a coverage ranging from 86.06% to 1.24%, whereas in India it covers up to 55.28% of the population. The epitope YPLLWSFAM had a coverage ranging from

TABLE 2 List of MHC class I alleles interacting with the epitopes predicted using IEDB MHC I prediction tool

Peptide sequence	HLA alleles ($IC_{50} < 500$)
	Phosphoprotein
VSDAKMLSY	HLA-A*01:01, HLA-A*29:02, HLA-A*30:02, HLA-C*12:03, HLA-C*05:01, HLA-C*14:02, HLA-C*07:01, HLA-C*08:02, HLA-C*06:02
PSDDFSNTF	HLA-C*12:03, HLA-C*05:01, HLA-B*15:02, HLA-C*07:01, HLA-C*15:02, HLA-A*01:01, HLA-C*07:02, HLA-C*06:02
REDLILPEL	HLA-C*12:03, HLA-B*40:01, HLA-B*40:02, HLA-C*05:01, HLA-C*08:02, HLA-C*07:02
MPKSRGIPI	HLA-C*12:03, HLA-B*07:02, HLA-B*08:01, HLA-C*03:03, HLA-B*15:02, HLA-B*53:01, HLA-C*14:02, HLA-B*35:01, HLA-B*51:01
ELRSELIGY	HLA-C*12:03, HLA-C*14:02, HLA-A*26:01, HLA-C*07:02, HLA-A*26:03, HLA-A*32:15, HLA-C*14:02, HLA-A*26:02, HLA-B*27:20, HLA-A*68:23, HLA-A*32:07
	Polymerase
ETDDYNGIY	HLA-C*12:03, HLA-A*01:01, HLA-C*14:02, HLA-A*26:01, HLA-B*15:02, HLA-C*07:01, HLA-A*30:02
NTSKSFLDY	HLA-C*03:03, HLA-C*12:03, HLA-A*29:02, HLA-A*01:01, HLA-B*15:02, HLA-C*14:02
EVDNNHLIY	HLA-C*05:01, HLA-C*12:03, HLA-A*01:01, HLA-B*35:01, HLA-A*29:02, HLA-B*15:02, HLA-C*14:02, HLA-C*07:01
LTPEMVLMY	HLA-C*12:03, HLA-A*29:02, HLA-C*14:02, HLA-A*01:01, HLA-B*15:01, HLA-A*26:01, HLA-B*35:01
RPRGRHTMV	HLA-B*07:02, HLA-C*12:03, HLA-B*08:01, HLA-A*68:23, HLA-A*32:15, HLA-A*32:07, HLA-A*02:50, HLA-B*27:20, HLA-B*40:13, HLA-B*42:01
	Nucleocapsid protein
EIISDIGNY	HLA-A*25:01, HLA-C*12:03, HLA-A*26:01, HLA-C*03:03, HLA-A*30:02, HLA-A*29:02
YPLLWSFAM	HLA-B*35:01, HLA-C*03:03, HLA-B*07:02, HLA-B*39:01, HLA-C*12:03, HLA-B*53:01, HLA-C*14:02, HLA-B*42:01, HLA-A*02:17, HLA-B*27:20, HLA-A*68:23, HLA-A*32:07.HLA-A*32:15, HLA-B*15:02, HLA-A*69:01, HLA-B*40:13, HLA-C*14:02, HLA-B*83:01, HLA-A*02:50
TIKSLMLLY	HLA-C*12:03, HLA-A*29:02, HLA-B*15:02, HLA-A*25:01, HLA-A*30:02, HLA-A*11:01, HLA-A*26:01, HLA-B*15:01
RRWAKYVQQ	HLA-C*Abb12:03, HLA-B*27:05, HLA-C*07:02, HLA-C*03:03, HLA-C*14:02
SRLAAKAAK	HLA-C*03:03, HLA-C*14:02, HLA-C*12:03, HLA-B*27:05, HLA-C*07:02, HLA-C*07:01, HLA-B*15:02

Abbreviations: IEDB, Immune Epitope Database; HLA, human leukocyte antigen; MHC I, major histocompatibility complex class I.

TABLE 3 Results of conservancy, toxicity, immunogenicity, and allergenicity analysis

Peptide sequence	Conservancy	Toxicity	Immunogenecity score	Allergenicity
Phosphoprotein				
VSDAKMLSY	43	Nontoxic	−0.435	Probable allergen
PSDDFSNTF	100	Nontoxic	0.005	Probable nonallergen
REDLILPEL	43	Nontoxic	0.164	Probable nonallergen
MPKSRGIPI	100	Nontoxic	−0.048	Probable nonallergen
ELRSELIGY	100	Nontoxic	0.070	Probable nonallergen
Polymerase				
ETDDYNGIY	100	Nontoxic	0.126	Probable allergen
NTSKSFLDY	100	Nontoxic	−0.318	Probable nonallergen
EVDNNHLIY	100	Nontoxic	0.093	Probable allergen
LTPEMVLMY	100	Nontoxic	−0.147	Probable nonallergen
RPRGRHTMV	100	Nontoxic	0.061	Probable nonallergen
Nucleocapsid protein				
EIISDIGNY	100	Nontoxic	0.048	Probable allergen
YPLLWSFAM	100	Nontoxic	0.168	Probable nonallergen
TIKSLMLLY	100	Nontoxic	−0.428	Probable nonallergen
RRWAKYVQQ	100	Nontoxic	−0.135	Probable allergen
SRLAAKAAK	94	Nontoxic	−0.073	Probable allergen

TABLE 4 Results for population coverage analysis of the best epitope in each of the three proteins

Population/Area	Phosphoprotein			Polymerase			Nucleocapsid protein		
	Coverage, %	Average hit	PC90	Coverage, %	Average hit	PC90	Coverage, %	Average hit	PC90
Central Africa	85.55	2.73	0.69	65.43	1.64	0.29	74.1	1.85	0.39
Central America	4.33	0.04	0.1	1.4	0.01	0.1	5.1	0.07	0.11
East Africa	82.69	2.38	0.58	66.41	1.64	0.3	68.05	1.5	0.31
East Asia	87.45	2.56	0.8	70.57	1.65	0.34	82.85	2.72	0.58
Europe	95.39	4.2	1.69	86.06	3.1	0.72	86.13	2.97	0.72
India	76.96	2.42	0.43	55.28	1.64	0.22	63.82	1.72	0.28
North Africa	88.58	3.16	0.88	71.44	2.22	0.35	70.74	1.88	0.34
North America	89.65	3.15	0.97	73.48	2.02	0.38	80.01	2.32	0.5
Northeast Asia	77.07	2.39	0.44	51.59	1.37	0.21	81.66	2.58	0.55
Oceania	79.35	2.16	0.48	47.27	1.11	0.19	65.94	1.74	0.29
South Africa	1.46	3.16	−839.18	1.24	2.25	−272.32	1.27	2.35	−297.35
South America	76.08	2.38	0.42	48.92	1.15	0.2	63.81	1.61	0.28
South Asia	80.8	2.73	0.52	58.88	1.85	0.24	69.19	1.97	0.32
Southeast Asia	77.69	2.26	0.45	39.87	1.0	0.17	77.68	2.21	0.45
Southwest Asia	83.0	2.68	0.59	58.1	1.71	0.24	62.82	1.64	0.27
West Africa	83.3	2.22	0.6	68.49	1.62	0.32	73.0	1.58	0.37
West Indies	72.49	1.21	0.36	62.58	1.32	0.27	61.76	1.14	0.26
World	90.47	3.45	1.04	75.85	2.38	0.41	80.63	2.56	0.52
Average	74.02	2.52	−46.01	55.71	1.65	−14.85	64.92	1.91	−16.16
Standard deviation	25.79	0.86	192.37	22.17	0.64	62.44	23.03	0.65	68.2

the highest 86.13% to the least 1.27% with 61.64% coverage in India. The complete coverage of populations for all three epitopes is given below in Table 4.

3.7 | Binding to MHC II alleles

Since MHC II binding is an important property to promote a complete immune response, the alleles binding to MHC class II were identified for these three epitopes and the results are displayed in Table 5. The polymerase epitope RPRGRHTMV did not bind to any of the MHC class II HLA alleles within the cutoff range.

3.8 | Molecular docking and dynamics simulations

The binding affinities of the HLA-C*12:03 as protein and the epitopes ELRSELIGY and YPLLWSFAM as peptides were calculated using AutoDock Vina and the binding energy was obtained in kcal/mol. The polymerase protein was not considered for docking because of its lower population coverage and had no MHC class II binding within the assigned IC_{50} value. The binding site residues obtained from CASTp 3.0 server were HIS188, VAL189, THR190, HIS191, HIS192, TRP204, LEU206, ARG234, GLN242, LEU272, ARG273, TRP274, GLU275, PRO276, and SER277 in A chain and TYR10, SER11, HIS13, PRO14, ALA15, GLU16, ASN17, GLU74, TRP95, ARG97, ASP98, and MET99 in B chain. Among the five predicted epitope structures, model 5 of epitope YPLLWSFAM had the least binding score of −8.8, −8.7, and −8.8 kcal/mol from the docking runs, respectively, whereas model 4 of epitope ELRSELIGY had the least binding energy of −7.9, −7.6, and 8.0 kcal/mol. These two epitope models were the best effective in all the three runs. The binding scores for all the models in each of the three runs are mentioned in Supporting Information Table 3. PatchDock results revealed that these two models had the highest score of 8482 and 9034, respectively. This was also consistent with the CABS-dock results with the maximum root-mean-square deviation (RMSD) value being 41.8 and 39.4. Figures 3 and 4 explain the molecular interaction of HLA-C*12:03 with epitopes ELRSELIGY and YPLLWSFAM, respectively.

The complex was further used to analyze the protein-peptide stability with molecular dynamic simulation for 100 nano second (ns).[76] From Figure 5A, the calculated RMSD for the peptide ELRSELIGY of phosphoprotein with HLA complex was stable after 65 ns of simulation, the average RMSD value was found to be ~0.38 Å. In contrast to that, the YPLLWSFAM of polymerase protein with HLA

TABLE 5 List of best epitopes and its associated MHC class II alleles

Protein	Peptide sequence	MHC II alleles
Phosphoprotein	ELRSELIGY	HLA-DRB1*01:01, HLA-DRB3*01:01
Nucleocapsid protein	YPLLWSFAM	HLA-DRB1*04:04, HLA-DRB1*15:01, HLA-DRB5*01:01, HLA-DRB1*04:01, HLA-DRB1*01:01, HLA-DRB1*07:01, HLA-DRB1*13:02, HLA-DRB1*09:01, HLA-DRB1*04:05, HLA-DPA1*03:01/DPB1*04:02

Abbreviations: HLA, human leukocyte antigen; MHC II, major histocompatibility complex class II.

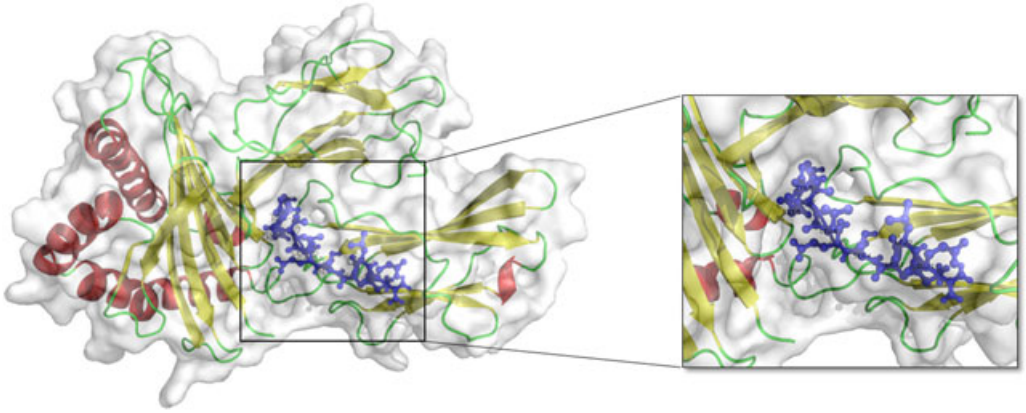

FIGURE 3 Molecular interaction between HLA-C*12:03 and the epitope ELRSELIGY of phosphoprotein. The protein is shown as cartoon surface structure and the binding epitope is shown as a blue ball and stick appearance. HLA-C, human leukocyte antigen-C

complex was stable after 40 ns until 100 ns and the calculated average RMSD value was found to be ~0.35 Å depicted in Figure 5B. Hence, the above results clearly indicate that there is a conformational change occurred after docking.

3.9 | B-cell epitope prediction

A sequential approach was carried out for the three proteins to predict possible B-cell epitopes. Based on all the predictions available for B-cell epitopes from IEDB, the sequence positions 421 to 471 in phosphoprotein, 606 to 640 in polymerase and 496 to 517 in nucleocapsid protein were identified as possible regions for B-cell epitopes. The BepiPred linear epitope predictions in Figure 6A-C show the probable epitope regions. Residues above the threshold are denoted in yellow and considered as most likely epitope region.

4 | DISCUSSION

Though the advancing medical technology aims to subsist with the current scenario of emerging viral disease, there has been no successful treatment or prevention choice available

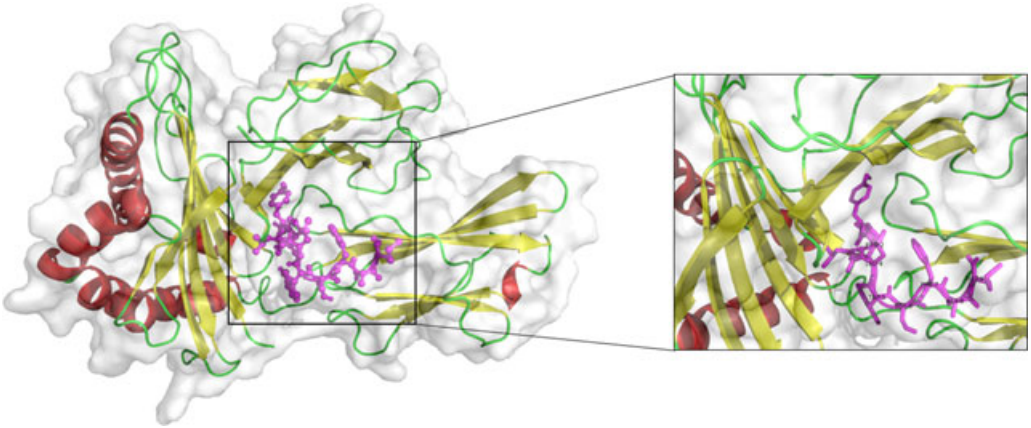

FIGURE 4 Molecular interaction between HLA-C*12:03 and the epitope YPLLWSFAM of nucleocapsid protein. The protein is shown as cartoon surface structure and the binding epitope is shown as a purple ball and stick appearance. HLA-C, human leukocyte antigen-C

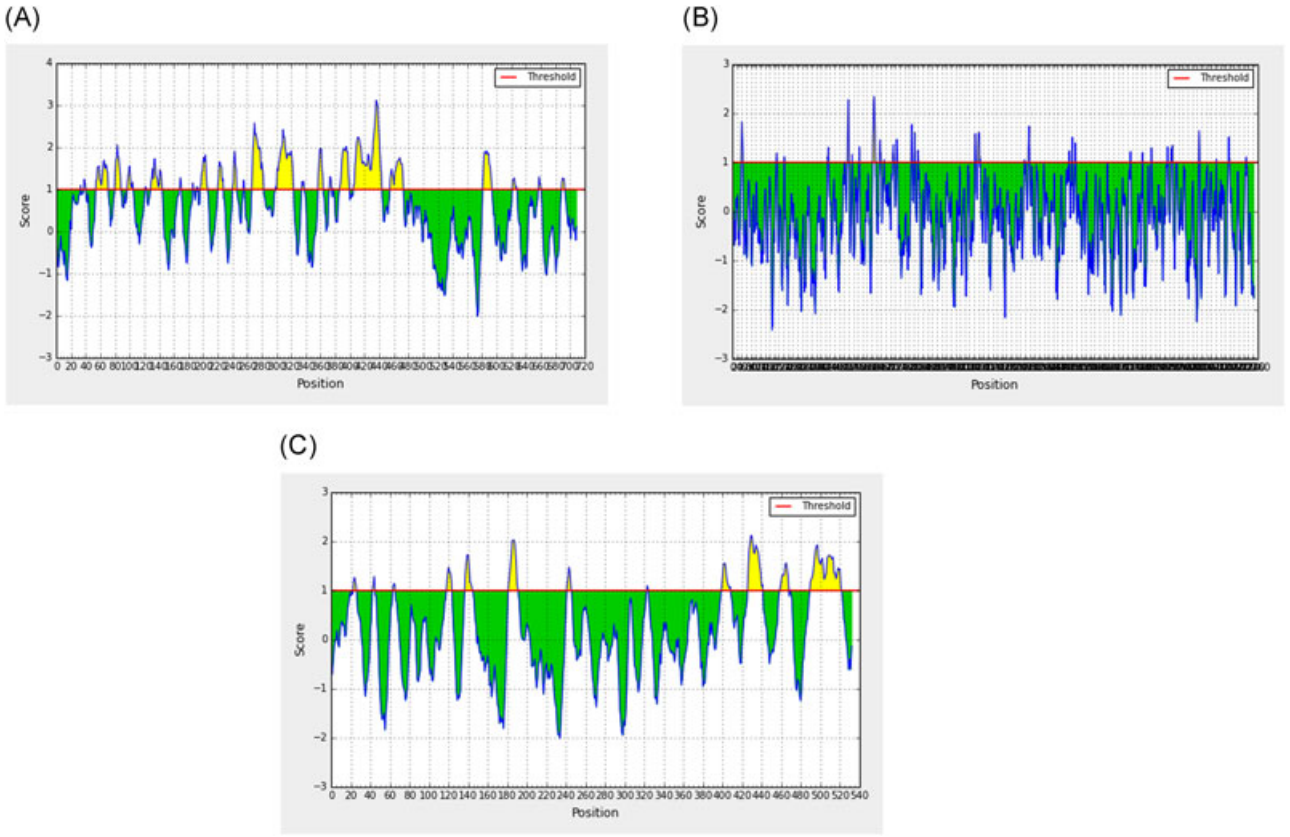

FIGURE 5 BepiPred linear epitope prediction showing probable epitope region of A, phosphoprotein; B, polymerase; and C, nulceocapsid protein. Threshold set was 1.000. X-axis represents position of the sequence and Y-axis indicates antigenic propensity. The regions denoted in yellow above the threshold level indicate probable regions of B-cell epitopes. RMSD, root-mean-square deviation

for NiV infection. However, the consistent NiV outbreaks with more than 600 affected patients and fatality rate up to 100%[77] creates an urge to develop an effective vaccine to manage future outbreaks. For over a decade, utilization of genomic data toward finding vaccine candidates is at a high pace in the domain of immunoinformatics which is trouble free, economical and has no safety issues.[78] We used tools to identify both T-cell and B-cell epitopes as the humoral immunity generated by the produced antibodies can eventually diminish but the T-cell-mediated immunity lasts longer.[79] This study availed the maximum number of criteria for a quality approach to obtain potential epitope candidates. There are considerable studies on epitope characterization for vaccine development that has its focus on glycoprotein and fusion protein. Nevertheless, there are no much reports on the antigenic properties of RdRp complex, a potential target, as it is the central unit of the viral replication and its encapsidation. Here we aimed to explore if the proteins of this complex possess immunogenic properties to be a part of vaccine-elucidated immune response.

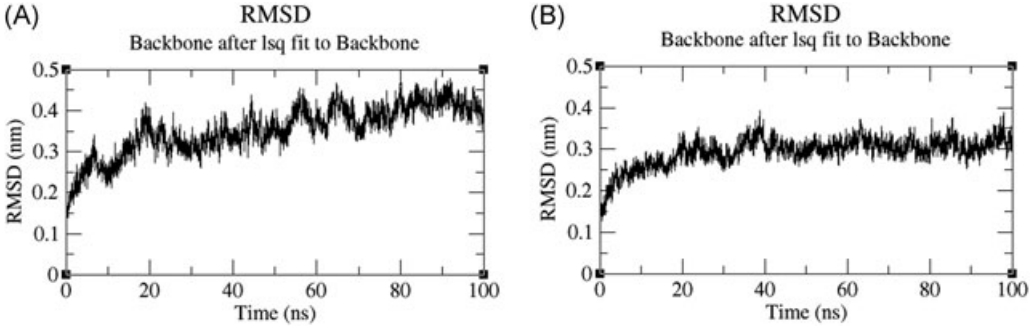

FIGURE 6 A, RMSD time evaluation of peptide ELRSELIGY with HLA complex; B, RMSD time evaluation of peptide YPLLWSFAM; HLA, human leukocyte antigen; RMSD, root-mean-square deviation

Three T-cell epitopes, ELRSELIGY in phosphoprotein, RPRGRHTMV in polymerase, and YPLLWSFAM in nucleocapsid protein was narrowed down from 31 protein sequences based on the analyzed vital properties. The different sequences of all three proteins had good conservancy of more than 90% which depicts these proteins can be used as a suitable prospect for the study. The best five epitopes obtained from the NetCTL-1.2 server were again evaluated using VaxiJen 2.0 as an effort to double check the antigenicity of individual epitopes. All our predictions were based on 9-mer selections for T-cell epitopes as T-cell antigenic peptides are made of 8 to 10 amino acids in length and bind to the host MHC groove. Though MHC I binding alone is considered in some studies[19,46] this study extends on identifying the MHC II alleles for the best three epitopes to assist quality design in every aspect. Out of the three epitopes, the polymerase epitope did not bind to any of the available MHC II alleles with an IC_{50} value below 500 nM. Hence, this epitope may not be potential to evoke a complete response to the NiV infection. In addition, we eliminated epitopes that were allergenic and nonimmunogenic as they might truly not ease the host with combatting the infection instead and aggravates the response. Population coverage analysis was restricted only to the MHC I alleles because not all MHC II alleles of the DRB region were available.[4] Interestingly we found that all the three proteins had the highest coverage in Europe. The average coverage of phosphoprotein and nucleocapsid protein were 77.65% and 77.67%, respectively, in Southeast Asia where the outbreaks are recurrent. However, the cumulative coverage of polymerase epitope in the Southeast Asian population is less than 50%. These results explain that epitope-based vaccine formulated using phosphoprotein and nucleoprotein could be applied to Southeast Asia, which is prone to outbreaks with special reference to India and very well to the European populations. These docking results and its validation with molecular dynamics simulation directly indicate that the T-cell epitopes ELRSELIGY and YPLLWSFAM with HLA undergo striking conformation changes promoting binding to form a thermodynamically stable complex. It is evident with our results that sequence positions of T-cell and B-cell epitopes do not overlap each other. However in comparison of this study, the epitope prediction of T cell is more reliable than B cell.[22] B-cell epitope prediction tools limit the utility because of its uncertainty for linear and conformational B-cell epitopes that cannot be isolated from their protein context.

5 | CONCLUSION

Establishing successful curatives or prevention is a high priority against Nipah viral infection and significant research is on progress. Although there is no licensed drug so far, vaccine-based approaches are advantageous in that they possess lesser pitfalls of drug resistance. Our study focuses on the RdRp complex to scan through these protein sequences identifying both T-cell and B-cell epitopes using various properties. We identified ELRSELIGY from phosphoprotein and YPLLWSFAM from nucleocapsid protein are the best-filtered T-cell epitopes. The sequence positions 421 to 471 in phosphoprotein, 606 to 640 in polymerase, and 496 to 517 in nucleocapsid protein are the best-predicted regions for B-cell epitopes. However, considering class II MHC binding and population coverage the polymerase protein may not be as potential as the epitopes of phosphoprotein and nucleocapsid protein. Considering prediction reliability, T-cell epitopes can form a concrete foundation to design a potential vaccine against Nipah virus. This can be further verified for solidity and efficacity with wet laboratory experimentation involving cell lines and animal studies followed by clinical trials.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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