Research Article

Epitope-Based Peptide Vaccine Construction Against Nipah Virus Using Comprehensive Immunoinformatics Approaches

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Abstract: Nipah virus (NiV) outbreaks continue to pose a serious public health threat in South Asia, particularly in India, with no approved vaccines currently available for human use. To address this challenge, we employed an integrated reverse vaccinology and immunoinformatics approach to design a multiepitope vaccine targeting the NiV G glycoprotein, a key viral surface protein responsible for host-cell attachment. Computational pipelines were utilized to predict antigenic B-cell and T-cell epitopes, evaluate their immunogenicity, and assess their binding affinity with common human HLA alleles through molecular docking. A promising epitope, AVGFLVRTEFKYNDS, was identified and its three-dimensional structure modeled and validated. Unlike previous NiV vaccine design studies that focused on conserved peptide prediction alone, this work integrates a comprehensive workflow combining antigenicity screening, structural modeling, and molecular interaction analysis to enhance vaccine precision. Overall, the findings demonstrate the potential of computational vaccinology to accelerate rational NiV vaccine development and lay the groundwork for future experimental validation.

Keywords: Nipah virus; epitope-based vaccine; immunoinformatics; antigen predictions; Molecular docking.

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Introduction

Nipah virus (NiV) is a zoonotic virus that causes severe and often fatal illness in humans [1]. It belongs to the Henipavirus genus in the Paramyxoviridae family and is primarily transmitted through direct contact with infected animals such as fruit bats (Pteropus species), pigs, contaminated food or via human-to-human transmission [2,3]. Outbreaks have been reported in South and Southeast Asia, including India, Bangladesh, and Malaysia, with high mortality rates and severe neurological and respiratory symptoms [3]. Due to the absence of licensed vaccines or specific antiviral treatments, NiV poses a serious public health threat, especially during seasonal outbreaks [4]. While various vaccine strategies are under research, including viral vector-based and subunit vaccines, none have yet achieved clinical approval. The limited progress and high risk associated with NiV infections highlight the urgent need to develop safe and effective vaccines against this deadly virus [5,6].

In recent years, immunoinformatics has emerged as a promising approach

to vaccine design using computational biology and bioinformatics tools to analyze pathogen genomes, predict antigenic epitopes, and design multiepitope vaccines that stimulate targeted immune responses [7]. This approach accelerates the early phases of vaccine discovery and enhances the potential to create broad-spectrum and safer vaccine candidates. Epitope-based vaccines, in particular, focus on specific regions of antigens recognized by the immune system, enabling the induction of both humoral (B cell) and cell-mediated (T cell) immune responses [8,9]. By targeting conserved epitopes, these vaccines can offer pathogen-specific and long-lasting protection with broad efficacy across multiple NiV strains.

The NiV genome, approximately 18,200 bases long, encodes six structural proteins (Nucleocapsid [N], Phosphoprotein [P], Matrix [M], Fusion [F], Glycoprotein [G], and Large [L] polymerase) which serve as potential vaccine targets [10,11]. Among the M, the G and F proteins are essential for viral entry into host cells and remain primary candidates for epitope prediction and vaccine formulation.

Severe infections can result in encephalitis and respiratory failure,, with case fatality rates ranging from 40% to 75% making NiV a WHO priority pathogen for urgent vaccine development [12]. Several candidates, including vector-based, subunit, and mRNA vaccines, are in preclinical or early clinical stages [5]. Notable examples include the ChAdOx1 NiV vaccine, based on a chimpanzee adenoviral vector expressing the NiV G glycoprotein, and the HeV-sG subunit vaccine, which cross-protects against NiV due to antigenic similarity with Hendra virus and is licensed for veterinary use in Australia [13]. However, these vaccines are yet licensed for humans and concerns remain about safety, efficacy, cross-reactivity, and immune-mediated complications upon natural infection.

A key limitation of many current vaccine strategies is their focus on structural proteins, often excluding non-structural proteins that contribute to immune modulation [14]. Henipaviruses, including NiV and Hendra virus, are genetically distinct yet highly conserved, with antigenic variation in glycoproteins influencing immune recognition and virulence. While G and F proteins mediate viral attachment and fusion, non-structural proteins such as the P, V, and W are critical for interferon antagonism and immune evasion [15]. Thus, identifying immunodominant protein sequences capable of eliciting durable immune responses is essential.

In this study, we employed computational immunoinformatics approaches to design multi-epitope-based vaccine candidates against the NiV. The constructs include predicted cytotoxic T lymphocyte (CTL) and helper T lymphocyte (HTL) epitopes, targeting multiple immune pathways to ensure robust adaptive responses [16]. Unlike conventional vaccines that rely on whole inactivated or attenuated viruses, epitope-based vaccines use short peptide sequences representing antigenic determinants. These can be chemically synthesized, offering a safer, more specific, cost-effective, and thermally stable alternative suitable for rapid responses to emerging viral

threats such as NiV [17, 18].

Similar computational strategies have been effectively applied in experimental vaccine design for Ebola virus, underscoring the versatility of this approach. However, there are still no commercially available epitope-based vaccines developed through immunoinformatics, emphasizing the need for translational research and clinical validation to adavance these innovative strategies for high-fatality zoonotic diseases like the NiV.

METHODOLOGY

Retrieval of Viral Protein Sequences

The structural sequence of Glycoprotein (G) of NiV was retrieved from the Uniprot database (https://www.uniprot.org) (accession number: Q9IH62) [19]. This sequence, consisting of 602 amino acids, was selected due to its potential relevance in vaccine design and immune response prediction.

T-Cell Epitope Prediction

The prediction of cytotoxic T-lymphocyte (CTL) epitopes was carried out using the Immune Epitope Database (IEDB) analysis resource, focusing on both MHC Class I and Class II molecules (http://tools.iedb.org/main) [20]. The full-length amino acid sequence of the NiV structural protein was submitted to the server, and predictions were performed across 31 selected human leukocyte antigen (HLA) Class I alleles. Peptides with a percentile rank of ≤ 0.3 were considered high-affinity binders and selected as potential T-cell epitopes.

Antigenicity Prediction

The antigenicity scores, after filtering B-cell and T-cell epitopes (MHC classes I & II) according to their respective thresholds, were predicted using the VaxiJen v2.0 server [21]. A threshold of 0.4 and above was used to select epitopes based on their physicochemical properties [22]. To identify the most effective antigenic protein, the antigenic value of each Nipah virus protein was determined using the VaxiJen v2.0 online server, which is the first alignment-independent server for predicting protective antigens [23]. The protein showing the highest antigenic score was considered the most effective antigenic protein.

Allergenicity and Toxicity Prediction

To ensure the safety and immunogenic suitability of the predicted epitopes, comprehensive allergenicity and toxicity analyses were performed. Allergenicity was evaluated using tools such as AllerTOP v.2.0, which employ machine learning algorithms and physicochemical property-based approaches to classify peptides as allergens or non-allergens [24]. Only those epitopes predicted to be non-allergenic by both platforms were selected for further analysis. Toxicity prediction was conducted using the ToxinPred server, which assesses potential toxic effects based on quantitative matrices and SVM-based models [25]. All selected epitopes were confirmed to be non-toxic, indicating a minimal risk of adverse immune reactions. These findings support the safe inclusion of the shortlisted epitopes in multi-epitope vaccine constructs against the Nipah virus.

Prediction of Peptide 3D Structure

To prepare for molecular docking and analyze the interactions between NiV epitopes and selected MHC alleles, the 3D structures of the predicted Nipah virus epitopes were generated using the PEP-FOLD 3 server [26]. This server provides possible 3D models for each peptide based on de novo structural modeling [27]. Among these, the most energetically favorable structure was selected for further simulations.

Molecular Docking

To identify the most promising vaccine candidates for Nipah virus, molecular docking of the MHC class I-interacting NiV epitopes was conducted using the ClusPro 2.0 server. Cluspro evaluates protein-peptide docking based on rigid body interaction models, scoring functions, and energy minimization [28]. This analysis allowed us to determine the binding orientation, interaction energy, and surface complementarity of each epitope-allele complex, thereby helping prioritize epitopes with high affinity and stable binding for further vaccine design considerations.

Population Coverage Analysis

After predicting the most interactive Nipah virus epitopes using ClusPro 2.0, population coverage analysis was conducted using the IEDB population coverage tool [29]. This analysis assessed the binding affinity of the selected NiV epitopes to MHC class I molecules across different ethnic and geographic populations worldwide, based on the HLA alleles associated with each epitope. A threshold of >80% population coverage was considered to identify epitopes with potential as broad-spectrum vaccine candidates [30].

RESULTS AND DISCUSSION

Nipah virus (NiV) continues to cause recurrent outbreaks with severe neurological and respiratory manifestations, often leading to high case fatality rates. The absence of approved vaccines underscores the urgent need for rational vaccine design strategies. In this study, we employed an immunoinformatics-based approach to identify and evaluate potential epitope candidates for the development of a peptide-based vaccine. This strategy offers a focused and accelerated alternative to conventional vaccine platforms by targeting conserved, non-allergenic, and highly antigenic regions of viral proteins capable of eliciting both humoral and cellular immune responses.

The glycoprotein (G) sequence of NiV was retrieved from the UniProt database in FASTA format and subjected to T-cell epitope prediction. A total of 100 epitopes each for MHC-I and MHC-II molecules were initially identified and subsequently screened based on antigenicity (VaxiJen score), allergenicity, and toxicity assessments. From this pipeline, two epitopes with the highest immunogenic potential were selected: MHC-I epitope MTRLAVKPK (VaxiJen score: 1.6597) (Table 1) and MHC-II epitope AVGFLVRTEFKYNDS (VaxiJen score: 1.4185) (Table 2). Population coverage analysis was carried out with particular emphasis on the Indian population, where the selected MHC-II epitope AVGFLVRTEFKYNDS demonstrated a coverage of 83.44%,

highlighting its relevance for a genetically diverse population (Fig. 1). To further assess its structural and interaction potential, the three-dimensional model of this epitope was generated using the PEP-FOLD3 server (Fig.2). Molecular docking studies were then performed with ClusPro, employing TLR-4 as the receptor protein obtained from the Protein Data Bank, since no experimentally resolved structure or reliable homology model was available for the relevant MHC-II allele (HLA-DPA1*01:03). TLR-4 was selected as it is a key receptor in innate immunity, recognizing pathogen-associated molecular patterns and initiating immune responses. The docking analysis produced a score of –112.4,

Table 1. Top 10 IEDB T-Cell (MHC Class I) Binding Prediction results of NiV and their Antigenicity, Allergenecity and Toxicity Prediction.

S.No	Epitope	Antigen Score	Antigen/Non-Antigen	Allergenecity	Toxicity
1	MTRLAVKPK	1.6597	Antigen	Non-Allergen	Non-Toxin
2	LLKNKIWCI	1.661	Antigen	Allergen	Non-Toxin
3	EIYDTGDNV	1.4043	Antigen	Allergen	Non-Toxin
4	TEIGPKVSL	1.4043	Antigen	Non-Allergen	Non-Toxin
5	EISDQRLSI	1.2708	Antigen	Allergen	Non-Toxin
6	NPKVVFIEI	1.136	Antigen	Allergen	Non-Toxin
7	VFIEISDQR	1.1813	Antigen	Allergen	Non-Toxin
8	NPKVVFIEI	1.136	Antigen	Allergen	Non-Toxin
9	KPKLISYTL	1.0819	Antigen	Non-Allergen	Non-Toxin
10	VFYQASFSW	1.0092	Antigen	Non-Allergen	Non-Toxin

Table 2. Top 10 IEDB T-Cell (MHC Class II) Binding Prediction results of NiV and their Antigenicity, Allergenecity and Toxicity Prediction

S.No	Epitope	Antigen Score	Antigen/Non-Antigen	Allergenecity	Toxicity
1	AVGFLVRTEFKYNDS	1.4185	Antigen	Non-Allergen	Non-Toxin
2	VVFIEISDQRLSIGS	1.4107	Antigen	Non-Allergen	Non-Toxin
3	PANIGLLGSKISQST	1.2797	Antigen	Non-Allergen	Non-Toxin
4	VRTEFKYNDSNCPIT	1.2016	Antigen	Allergen	Non-Toxin
5	TVNPLVVNWRNNTVI	1.1349	Antigen	Allergen	Non-Toxin
6	KQRIIGVGEVLDRGD	1.0679	Antigen	Non-Allergen	Non-Toxin
7	TMDIKKINEGLLDSK	1.0167	Antigen	Non-Allergen	Non-Toxin
8	SRGVSKQRIIGVGEV	1.0135	Antigen	Non-Allergen	Non-Toxin
9	TLYFPAVGFLVRTEF	1.001	Antigen	Non-Allergen	Non-Toxin
10	DKVMPYGPSGIKQGD	0.9907	Antigen	Non-Allergen	Non-Toxin

indicating a stable binding affinity between the epitope and TLR-4 (Fig.3). The findings collectively demonstrate the feasibility of employing computational pipelines to screen, validate, and model NiV epitopes for vaccine design. While these results provide promising leads, experimental validation through in vitro immunogenicity assays and in vivo animal studies will be essential to confirm the protective potential of these epitopes.

MHC class	Coverage	Average hit	PC90
II	83.44%	0.83	0.6

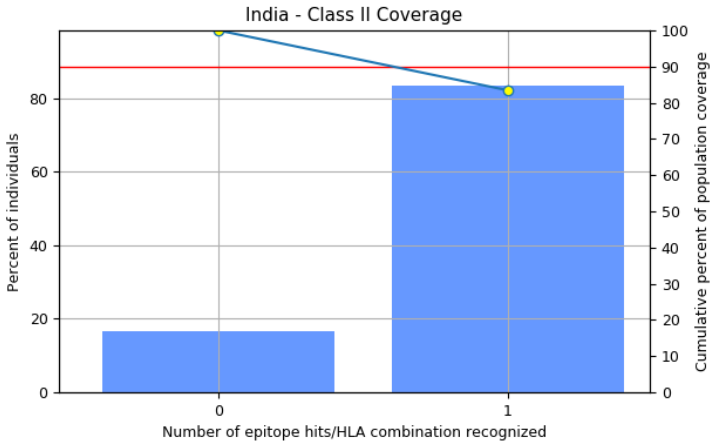

Figure 1: Population coverage analysis of the selected T-cell epitope (AVGFLVRTEFKYNDS) revealed a combined MHC class coverage of 83.4%, with an average of 0.83 epitope hits per individual and a PC90 value of 0.6

Figure 2: PEP-FOLD3 predicted 3D structure of the epitope

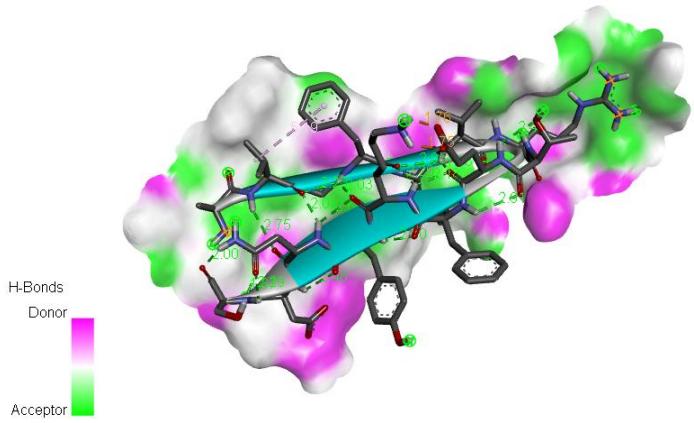

Figure 3: Docking complex and molecular interaction between TLR-4 and the epitope AVGFLVRTEFKYNDS.

Future directions could also include designing a multi-epitope construct incorporating both CTL and HTL epitopes, coupled with suitable adjuvants and delivery systems, to maximize immunogenicity and ensure long-lasting protective immunity against Nipah virus infections.

CONCLUSION

This work focused on the epitope-based immunoinformatics design of a vaccine against Nipah virus (NiV). Despite the challenges of conducting NiV research - owing to its requirement for biosafety level 4 (BSL-4) containment, high mortality rate, and epidemic potential - it remains a critical threat to global health. The predicted epitope identified in this study may serve as a valuable foundation for the development of peptide-based vaccines against NiV. Moreover, the common antigenic peptides identified here could play a pivotal role in designing synthetic peptide vaccines with improved safety and specificity. Future research should focus on in vitro immunogenicity assays, molecular dynamics simulations, and in vivo evaluations in suitable animal models to validate the immunoprotective potential of these epitopes. Collectively, these findings provide a rational framework for advancing Nipah virus vaccine research from computational prediction to experimental realization.

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