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Reverse Vaccinology Analysis of B-cell Epitope against Nipah Virus using Fusion Protein

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ABSTRACT

Nipah virus (NiV) is an RNA virus, a pathogenic paramyxovirus that causes nonlethal respiratory illness in pigs. It was originally reported in Malaysia in 1998. NiV is considered a potential outbreak threat because it is zoonotic. However, no vaccines or antiviral drugs have been found against NiV. Therefore, the main objective is to develop effective vaccines by characterizing the fusion protein of NiV. We used a reference sequence retrieved from the National Center for Biotechnology Information (NCBI), then 3D modeled it to obtain the conserved region of the fusion protein. The interaction between the conserved region and B-cell receptors has been evaluated through a molecular docking approach. The B-cell epitope was identified using the Immune Epitope Database (IEDB) web server. As a result, we recommend Pep_D FANCISVTCQCQ as an epitope-based peptide vaccine candidate against Nipah virus. Pep D is highly immunogenic and does not cause autoimmune reactions. Pep D has the lowest binding energy for BCR molecular complexes, which can activate the transduction signal and direct B-cell immune response. However, further studies are required for confirmation (in vitro and in vivo).

Keywords: Fusion protein, Nipah virus, reverse vaccinology, immunoinformatic.

INTRODUCTION

Nipah virus (NiV) is an RNA virus, a pathogenic paramyxovirus originally reported in Malaysia in 1998, primarily causing nonlethal respiratory illness in pigs [1]. NiV is highly pathogenic to a wide range of mammals due to its zoonotic transmission (from bats to humans, or from bats to pigs and then to humans) as well as human-to-human transmission [2]. Therefore, this virus has the potential to cause outbreaks.

Nipah virus belongs to the Paramyxoviridae family and is the second member of the Henipavirus genus. The virus is named after Kampung Sungai Nipah, a village in Negeri Sembilan where pig farmers were found to have encephalitis [3].

Human-to-human transmission is estimated to be prevalent in India and Bangladesh, accounting for 75% and 51% of cases, respectively, in 2004. Currently, there are no vaccines or antiviral drugs available for NiV disease, with the only treatment being supportive care [4]. To prevent further infections, the development of effective vaccines and/or therapeutics is indeed a necessity at this time.

A number of vaccine candidates have demonstrated complete protection against NiV disease in preclinical testing using small animal and nonhuman primate models. Additionally, several NiV vaccine trials have been

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conducted, with the NiV envelope proteins F (fusion) and G (glycoprotein) selected for vaccine development based on our previous understanding of immunity to other paramyxoviruses [5].

There are currently several immunoinformatic techniques available for predicting B and T cell epitopes with excellent sensitivity and specificity. These techniques are essential for understanding the molecular basis of immunity and for developing epitope-based peptide vaccines. In this study, we employed reverse vaccinology analysis to predict B-cell epitopes for vaccine development against the *Nipah virus*.

MATERIAL AND METHODS

Sample Retrieval from Database

The NiV fusion (F) protein (RefSeq. NP_112026.1) was obtained from the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/) [6].

The Screening of Conserved Domain

The 3D structure of the conserved domain in the NiV fusion protein sequences was modeled using SWISS-MODEL (https://swissmodel.expasy.org) with a homology modeling approach [7].

Immunoinformatics Prediction

This study involved the prediction of linear B-cell epitopes using tools such as BepiPred, Emini Surface Accessibility, and Kolaskar-Tongaonkar antigenicity available on the Immune Epitope Database Analysis Resource (IEDB-AR) webserver (http://tools.iedb.org/main/bcell) [8]. We predicted the characteristics of candidate epitopes that can act as protective antigens using the VaxiJen v2.0 web server (http://www.ddgpharmfac.net/vaxijen/VaxiJen/VaxiJen.html) [9].

The toxicity and allergenicity of the peptides were predicted using the ToxinPred online tool (http://crdd.osdd.net/raghava/toxinpred/) and the AllerTOP v2.0 tool (https://www.ddg-pharmfac.net/AllerTOP/), respectively. These tools were also employed to predict the toxicity and allergenicity of the bioactive peptides. [9,10]. Prediction of similarity with proteins in Homo sapiens cells is performed on vaccine candidate peptides using the Basic Local Alignment Protein Search Tool (BLASTp) (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Protein Docking

The protein docking process was employed to determine the molecular interaction between the ligand and the receptor. In this study, the candidate epitope from the NiV fusion (F) protein was bound to B-cell receptors (BCR). The receptor was obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) (https://www.rcsb.org/) [12]. Peptides must be converted into PDB format before their structures can be predicted using the PEPFOLD-3 webserver https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3) [13]. PatchDock is also used to predict the structure of protein–protein and protein–small molecule complexes.(https://bioinfo3d.cs.tau.ac.il/PatchDock/php.php) [14].

Docking is a technique for elucidating fundamental biological processes and characterizing the behavior of small molecules in the binding sites of target proteins. [15]. The results were visualized in PyMol software to provide a three-dimensional (3D) representation of the proteins. [16].

Results and Discussion

Conserved Identification from NiV fusion protein

A fusion protein of Nipah virus sequences was used in this study, and it had previously been obtained from the NCBI web server. The reference sequences are protein sequences from Nipah henipavirus. The sequences, which have a length of 546 amino acids, have been obtained from NCBI and can be seen as follows:

"MVVILDKRCYCNLLILILMISECSVGILHYEKLS
KIGLVKGVTRKYKIKSNPLTKDIVIKMIPNVSNMSQ
CTGSVMENYKTRLNGILTPIKGALEIYKNNTHDLVG
DVRLAGVIMAGVAIGIATAAQITAGVALYEAMKNA
DNINKLKSSIESTNEAVVKLQETAEKTVYVLTALQD

YINTNLVPTIDKISCKQTELSLDLALSKYLSDLLFVF
GPNLQDPVSNSMTIQAISQAFGGNYETLLRTLGYAT
EDFDDLLESDSITGQIIYVDLSSYYIIVRVYFPILTEIQ
QAYIQELLPVSFNNDNSEWISIVPNFILVRNTLISNIEI
GFCLITKRSVICNQDYATPMTNNMRECLTGSTEKCP
RELVVSSHVPRFALSNGVLFANCISVTCQCQTTGRA
ISQSGEQTLLMIDNTTCPTAVLGNVIISLGKYLGSVN
YNSEGIAIGPPVFTDKVDISSQISSMNQSLQQSKDYI
KEAQRLLDTVNPSLISMLSMIILYVLSIASLCIGLITFI
SFIIVEKKRNTYSRLEDRRVRPTSSGDLYYIGT"

The sequences were modeled using the SWISS-MODEL web server. Three-dimensional (3D) modeling is required to identify the structure of the protein to be utilized, which will subsequently assist in the identification process.

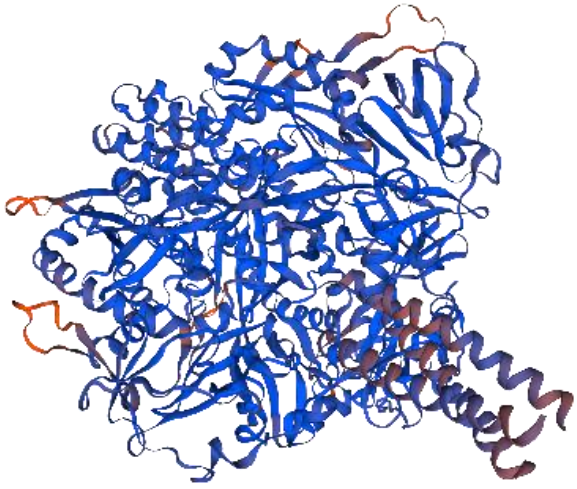

Fig. 1. The conserved domain on fusion proteins was analyzed for the potential of B-cell immunogenicity to obtain specific 3D structure of fusion protein Nipah virus modelled using SWISS-MODEL.

The B-cell Immunogenicity Predictions of Peptide Vaccine Candidate

The conserved domain on fusion proteins was analyzed for the potential of B-cell immunogenicity to obtain specific peptides as vaccine candidates. In our study, we predicted the B-cell epitopes of the conserved region of the fusion protein based on BepiPred, Emini Surface Accessibility, and Kolaskar–Tongaonkar antigenicity using the IEDB.

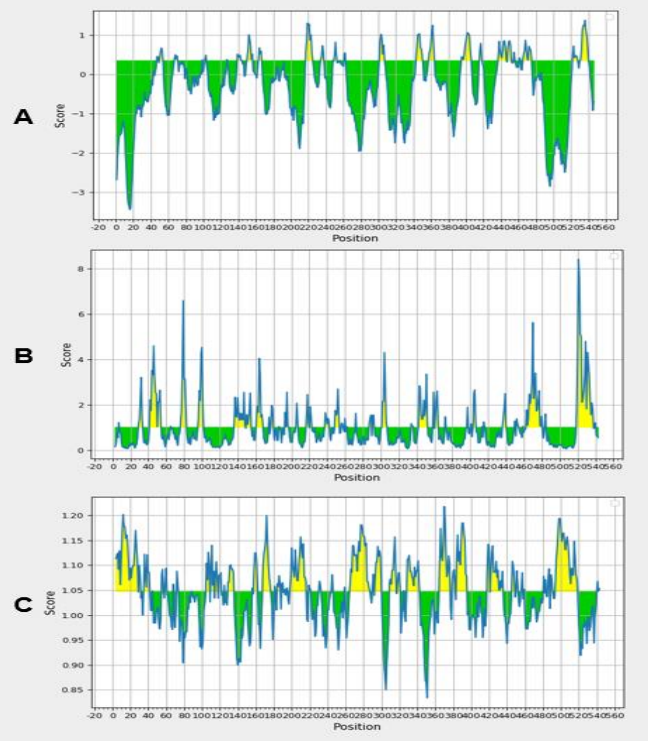

Fig. 2. Prediction of B cell epitopes by different parameters. (A) BepiPred, (B) Emini surface accessibility, (C) Kolaskar–Tongaonkar antigenicity. B cell epitope prediction was done using the IEDB server. The yellow region was a positive prediction of B cell epitope, whereas the green region was negative.

These methods were used to predict specific areas in proteins that bind to the B cell receptor, and these areas must be on the surface and immunogenic (Fig. 2). Bepipred was used to predict linear B-cell epitopes, resulting in 4 epitopes with a minimum propensity of -0.006, a maximum score of 1.381, and a threshold of 0.350. Emini Surface Accessibility was used to predict the surface accessibility of a protein, yielding 8 peptides with a minimum propensity of 0.047, a maximum score of 8.418, and a threshold of 1.000. Kolaskar-Tongaonkar was used to predict antigenic determinants on the protein, resulting in 5 peptides with a minimum propensity of 0.834, a maximum score of 1.218, and a threshold of 1.047. We predicted the antigenicity of the peptides using VaxiJen v2.0 and obtained peptides with antigenic properties. Furthermore, the peptides were analyzed for their similarity to cell surface receptors in Homo sapiens using the BLASTp server.

TABLE I. PREDICTION OF B CELL EPITOPES USING BEPIPRED

No	Peptide	VaxiJen 2.0	AllerTOP	ToxinPred	Similarity
1	QTTGRAISQSGE	Antigen	Non-Allergen	Non-Toxin	< 40
2	QSLQQSKDYIK	Non - Antigen	Non-Allergen	Non-Toxin	No Similaritites
3	NYNSEGIAIG	Antigen	Allergen	Non-Toxin	No Similaritites
4	RRVRPTSSGD	Antigen	Allergen	Non-Toxin	<40

TABLE II. PREDICTION OF B CELL EPITOPES USING EMINI SURFACE ACCESSIBILITY

No	Peptide	VaxiJen 2.0	AllerTOP	ToxinPred	Similarity
1	MIILYVLSIASLCIGLITFISFIIV	Antigen	Non-Allergen	Non-Toxin	80-200
2	SLDLALSKYLSDLLFVFGP	Non - Antigen	Non-Allergen	Non-Toxin	< 40
3	FANCISVTCQCQ	Antigen	Allergen	Non-Toxin	< 40
4	ILDKRCYCNLLILILMISECSVGIL	Antigen	Allergen	Non-Toxin	< 40
5	QIIYVDLSSYYIIVRVYFPILTE	Non - Antigen	Non-Allergen	Non-Toxin	No Similaritites
6	GFCLITKRSVICNQ	Antigen	Allergen	Non-Toxin	No Similaritites
7	LGNVIISLGKYLGS	Non - Antigen	Non-Allergen	Non-Toxin	40 - 50
8	RELVVSSHVPRF	Antigen	Non-Allergen	Non-Toxin	< 40

TABLE III. PREDICTION OF B CELL EPITOPES USING KOLASKAR – TONGAONKAR ANTIGENICITY

No	Peptide	VaxiJen 2.0	AllerTOP	ToxinPred	Similarity
1	EKKRNTYSRLEDRRVRPTS	Antigen	Non-Allergen	Non-Toxin	50 - 80
2	MNSQSLQQSKDYIKEAQRL	Non - Antigen	Non-Allergen	Non-Toxin	No Similaritites
3	VTRKYIKSNPLT	Antigen	Allergen	Non-Toxin	No Similaritites
4	EAMKNADNINKLK	Non-Antigen	Allergen	Non-Toxin	40 - 50
5	QDYATPMTNNM	Non-Antigen	Allergen	Non-Toxin	No Similaritites

The screening identified B-cell epitopes of NiV fusion protein peptides that met the criteria for vaccine candidates, and these epitopes are highlighted in Table IV. These candidates can be further evaluated using a molecular docking method for selection.

TABLE IV. PEPTIDES CANDIDATE

Molecular Complex	Peptide	Receptor	Global Energy (kcal/mol)
Pep_A	QTTGRAISQSGE	5IFH	-48.18
Pep_B	MIILYVLSIASLCIGLITFISFIIV	5IFH	-47.05
Pep_C	SLDLALSKYLSDLLFVFGP	5IFH	-34.17
Pep_D	FANCISVTCQCQ	5IFH	-49.70
Pep_E	EKKRNTYSRLEDRRVRPTS	5IFH	1.24

The peptides showed no similarity to the cell surface receptors of Homo sapiens, with a score of less than 20%, suggesting that they are unlikely to cause autoimmune reactions [17].

Molecular Interaction between Peptide-BCR

Peptides meeting the criteria for B-cell immunogenicity were modeled via PEP-FOLD using fold recognition [18]. Subsequently, the structures were obtained and stored in .pdb format. Next, 3D samples of the antigen-binding fragment BCR receptor (ID 5IFH) were retrieved from the RCSB database. Protein-peptide docking simulations using PatchDock were performed to determine binding energy, which is crucial for forming stable complexes and activating biological responses at BCR receptors[14]. The results display the binding energy generated by all the ligands and are visualized in PyMol software using structural and color selection (Fig. 3).

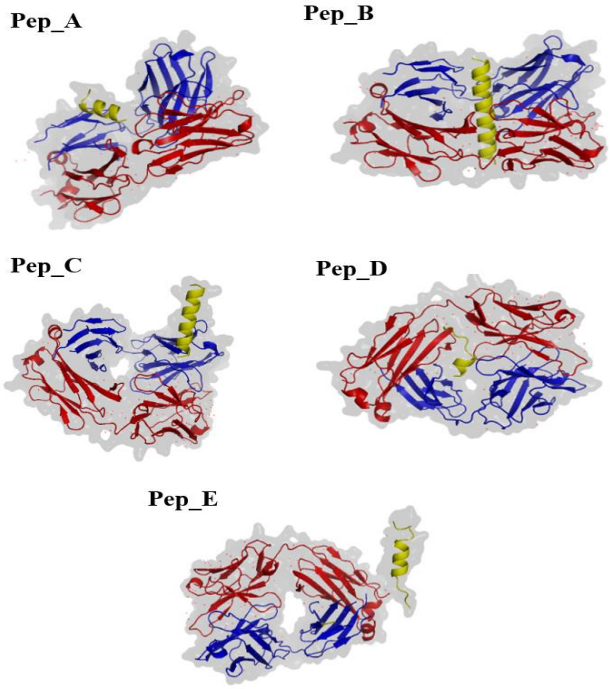

Fig. 3. Visualization of the bond between the ligand and the receptor of peptides candidate

The analysis revealed that Pep_D FANCISVTCQCQ is the most promising vaccine candidate against the Nipah virus. Pep_D holds significant potential as an epitope-based peptide vaccine candidate due to its exceptionally low binding energy score, facilitating the formation of molecular complexes [19,20,21,22].

CONCLUSION

In conclusion, the analysis of B-cell epitopes in the fusion protein NiV peptides has identified five candidates with varying global energy values. We recommend Pep_D FANCISVTCQCQ as a promising epitope-based peptide vaccine candidate against the Nipah virus due to its high immunogenicity and its non-triggering of autoimmune mechanisms. Pep_D has demonstrated the ability to form molecular complexes with the lowest binding energy, facilitating transduction signal activation and direct activation of the B-cell immune response. Nevertheless,

further research into NiV vaccine development should prioritize conducting in vivo and in vitro studies to validate the vaccine's performance.

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تحليل اللقاحات العكسي لخلايا B ضد فيروس نيباه باستخدام بروتين الانصهار

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ملخص

فيروس نيباه (NiV) هو فيروس باراميكسوفيروس ممرض لفيروس الحمض النووي الريبي ويسبب أمراضًا تنفسية غير مميتة في الخنازير التي تم الإبلاغ عنها في الأصل في ماليزيا في عام 1998. ويعتبر فيروس نيبا سببًا محتملاً لتفشي المرض لأنه حيواني المنشأ. ومع ذلك، لا توجد لقاحات أو عقاقير مضادة للفيروسات موجودة ضد النيكل. لذلك، فإن الهدف الرئيسي هو تطوير لقاحات فعالة من خلال توصيف بروتين الاندماج من NiV. استخدمنا التسلسل المرجعي الذي تم استرداده من المركز الوطني لمعلومات التكنولوجيا الحيوية ((NCBI، ثم تم تصميمه بنمذجة ثلاثية الأبعاد للحصول على المنطقة المحفوظة لبروتين الاندماج. تم تقييم التفاعل بين المنطقة المحفوظة مع مستقبلات الخلايا البائية من خلال نهج الالتحام الجزيئي. تم التعرف على حاتمة الخلية B باستخدام خادم الويب لقاعدة البيانات الحلقية المناعية (IEDB). نتيجة لذلك، نوصي بـ Pep_D FANCISVTCQCQ باعتباره لقاح ببتيد قائم على الحاتمة ضد فيروس نيباه. يعتبر Pep D عالي المناعة ولا يسبب تفاعلات المناعة الذاتية. يحتوي Pep D على أقل طاقة ملزمة للمجمعات الجزيئية BCR التي يمكنها تنشيط إشارة التحويل والاستجابة المناعية المباشرة للخلايا B. ومع ذلك، هناك حاجة إلى مزيد من الدراسة للتأكيد (في المختبر وفي الجسم الحي).

الكلمات الدالة: بروتين الاندماج، فيروس نيباه، التطعيم العكسي، المعلومات المناعية.

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