Original Article

Analysis of Dengue Virus Type 3 Isolate DENV3-5_S_162 Polyprotein-Based Vaccine Candidate: In Silico Study

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Abstract

Dengue Hemorrhagic Fever (DHF) is a serious infectious disease that warrants close attention due to its potential to progress rapidly into a life-threatening condition. It is commonly found in endemic areas and is often responsible for outbreaks. Among the four known Dengue virus serotypes, serotype 3 is most frequently associated with severe infections and serious complications in affected patients. The purpose of this study was to design a candidate dengue virus vaccine targeting dengue virus type 3 isolate DENV3-5_S_162 based on polyprotein. The characteristics of the resulting peptides are determined by their antigenicity, allergenicity, toxicity, instability and immunogenicity. The method used in this study is bioinformatics by utilizing several websites, namely the National Center for Biotechnology Information (NCBI) database, IEDB analysis resource, VaxiJen v2.0, AllerTOP v.2.0, ToxinPred, Protparam and Swiss model. The results of the application analysis showed that there were 2 potential peptides for dengue virus type 3 vaccine candidates, namely the GDQHQVGNETQGVTAEITPQASI peptide and the VEPGKNPKNFQTMPGTFQTTTGEI peptide. The peptide meets and has been tested with various stages such as antigenicity, allergenicity, toxicity, instability index and immunogenicity tests. The prediction results can be used as a reference for making a peptide vaccine for dengue virus type 3 isolate DENV3-5_S_162 with added value in terms of disease specificity, purity, production capacity, and production cost efficiency.

Keywords : *bioinformatics, in silico, peptides, dengue virus, vaccines.*

Abstrak

Demam Berdarah Dengue (DBD) adalah salah satu penyakit infeksi yang harus diwaspadai karena dapat berkembang menjadi penyakit yang fatal dengan cepat. Penyakit ini sering menyebar di daerah yang endemis dan sering menyebabkan wabah. Dari keempat serotipe virus Dengue yang diketahui, serotipe 3 paling sering berhubungan dengan infeksi berat dan komplikasi serius pada pasien. Tujuan penelitian ini untuk merancang kandidat Vaksin virus demam berdarah dengan menargetkan virus dengue type 3 isolate DENV3-5_S_162 berbasis polyprotein. Karakteristik peptida yang dihasilkan ditentukan sifat antigenisitas, alergenisitas, toksisitas, instabilitas dan immunogenisitasnya. Metode yang digunakan pada penelitian ini adalah bioinfomatika dengan memanfaatkan beberapa web, yaitu National Center for Biotechnology Information (NCBI) database, IEDB analysis resource, VaxiJen v2.0, AllerTOP v.2.0, ToxinPred, Protparam dan Swiss model. Hasil analisis aplikasi menunjukkan bahwa terdapat 2 peptida yang berpotensi untuk kandidat vaksin virus demam berdarah tipe 3 yaitu peptida GDQHQVGNETQGVTAEITPQASI dan peptida VEPGKNPKNFQTMPGTFQTTTGEI. Peptida tersebut memenuhi dan telah diuji dengan berbagai tahap seperti uji antigenisitas, alergenisitas ,toksisitas, instability indeks dan immunogenisitas. Hasil prediksi dapat dijadikan sebagai referensi pembuatan vaksin peptida untuk virus demam berdarah tipe 3 isolate DENV3-5_S_162 dengan memiliki nilai lebih dari sisi spesifisitas penyakit, kemurnian, kapasitas produksi, dan efisiensi biaya produksi.

Keywords : *bioinformatika, in silico, peptida, virus dengue, vaksin*.

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Introduction

Dengue Hemorrhagic Fever is a dangerous infectious disease that can cause death in a short time and often causes outbreaks[1]. The disease was first discovered in Manila, Philippines in 1953 and then spread to various countries. In Indonesia, the disease was first reported in 1968 in Surabaya. with 58 sufferers and 24 deaths (41.3%), but virological confirmation was only obtained in 1972 [2]. Furthermore, since then, Dengue Hemorrhagic Fever has tended to spread throughout Indonesia, so that by 1980 all provinces in Indonesia except East Timor had been infected with the disease, and reached its peak in 1988 with an incidence rate of 13.45% per 100,000 population. This condition is closely related to the increasing mobility of the population and in line with the increasingly smooth transportation relations[3].

One of the endemic illnesses in the tropics and certain subtropics is dengue hemorrhagic fever (DHF). Because it may spread swiftly throughout a region, the disease spread by the Aedes aegypti mosquito is a terrifying threat. In endemic places, the number of DHF cases might approach hundreds of dengue virus-infected individuals in a single month[4]. Acute dengue fever is characterized by bleeding symptoms that might result in shock and death. One of the four virus serotypes belonging to the genus Flavivirus and family Flaviviridae is responsible for dengue disease. Dengue fever comes in four different serotypes: Dengue 1, 2, 3, and 4. The predominant serotype that causes severe infections is Dengue type 3. The virus must incubate in the human body for four to six days (intrinsic incubation period) before it can cause illness[5]. Studies showed that Dengue type 3 is the dominant serotype of the virus that causes severe cases[6]. The Bioinformatics approach is considered the best method in designing modern vaccines, because it provides guidance on the classic trial and error method in a wet laboratory [7]. A peptide vaccination is one that is made up of many amino acid residues, often nine to fifteen. Protein is the primary component of the peptide vaccine candidate since amino is a monomer of protein[8]. To develop a peptide vaccine, bioinformatics methods can be used. The peptides produced from the bioinformatics prediction come from proteins contained in the virus/antigen, so that the predicted peptides can be used as peptide vaccine candidates [9].

Our study is expected to open a new dimension in developing a Polyprotein-based vaccine regimen for the Dengue Fever Virus Vaccine Candidate type 3 isolate DENV3-5_S_162. Besides, there has never been an in silico study of The Immune Epitope Database Analysis Resource (IEDB AR) in predicting the Dengue Fever Virus Vaccine Candidate type 3 isolate DENV3-5_S_162 based on Polyprotein itself. Therefore, the purpose of this study is to design a candidate for the Dengue Fever Virus Vaccine type 3 isolate DENV3-5_S_162 based on Polyprotein which is then simulated through binding testing using IEDB AR. The characteristics of the resulting peptide are determined by antigenicity, allergenicity, toxicity, instability index and immunogenicity. The prediction results can be used as a reference for making a peptide vaccine for dengue fever virus type 3 isolate DENV3-5_S_162 with added value in terms of disease specificity, purity, production capacity, and production cost efficiency.

Methods

Sample Collection from Database

The protein sequence of Dengue virus type 3 isolate DENV3-5_S_162 was retrieved from the National Center for Biotechnology Information (NCBI) database, with

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the GenBank accession number UDI89639.1. The sequence was prepared in FASTA format for further analysis.

Domain Screening

Domains within the DENV3-5_S_162 sequence were identified through sequence alignment using the NCBI database. The 3D structure of the aligned protein sequence was modeled through homology modeling using the Swiss Model web server. The resulting 3D structures were visualized and analyzed using PyMol software.

Immunoinformatics Prediction

The protein sequence in FASTA format was analyzed using the IEDB Analysis Resource. Initially, B-cell epitope prediction was conducted by selecting the Prediction of Linear Epitopes from Protein Sequence option. The BepiPred linear epitope prediction method was applied to identify peptides suitable for further evaluation. Selected peptides were subjected to the following tests: (1) Antigenicity Analysis: Using VaxiJen v2.0 to determine the antigenicity of the peptides. (2) Allergenicity Analysis: Using AllerTOP v.2.0 to assess potential allergenicity. (3) Toxicity Analysis: Using ToxinPred to evaluate peptide toxicity. (4) Stability Assessment: Using ProtParam to calculate the instability index of the peptides. (5) Finally, peptides were further analyzed using the IEDB Analysis Resource's ElliPro tool for epitope prediction based on structural protrusion. This method predicts epitopes considering solvent accessibility and flexibility, enabling the identification of structurally favorable candidates. The selected peptides' 3D structures were validated using the Swiss Model, ensuring structural integrity and compatibility for further immunological studies.

Results

Determining Candidate Domains

Aedes aegypti and Aedes albopictus mosquitoes are the main vectors of the Dengue virus (DENV), a single-stranded RNA virus belonging to the Flaviviridae family. This study focused on the DENV type 3 isolate DENV3-5_S_162 protein, identified using GenBank accession number UDI89639.1.

Epitope Candidate Prediction

Epitope candidates were predicted using the BepiPred linear epitope prediction tool in the IEDB Analysis Resource. This method identified linear regions in the protein sequence with high potential to interact with B-cells. Selected epitopes were based on their scores and structural accessibility. Figure 1 maps the predicted epitopes onto the 3D structure of the protein. Based on the analysis of the IEDB analysis resource processing, three main peptides were produced which are suspected to be vaccine candidates (Table 1).

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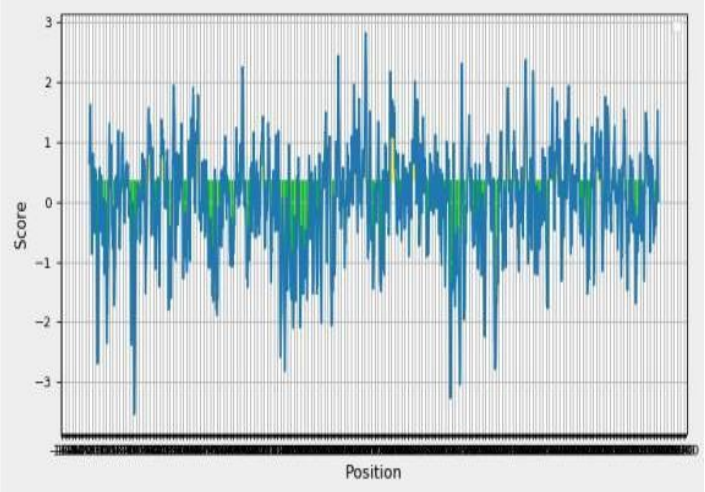

Figure 1. Predicted epitopes mapped onto the DENV3-5_S_162 protein using IEDB AR

Table 1. List of Candidate Epitopes and Their Sequences

Candidate Epitope	Sequence
Peptide 1	NITTDSRCPTQGEAVLPEEQDQN
Peptide 2	GDQHQVGNETQGVTAEITPQASI
Peptide 3	AGPISQHNHRPGYHTQTAGPW
Peptide 4	VEPGKNPKNFQTMPGTFQTTTGEI
Peptide 5	VVTKKEEPVNIEAEPPFGE

Antigenicity and Allergenicity Test

Antigenicity is the ability of an antigen to stimulate the formation of specific antibodies[10]. While allergenicity is the ability of a substance to cause allergies[11]. The antigenicity of the predicted peptides was evaluated using the VaxiJen v2.0 tool. Peptides with antigenicity scores above the threshold were considered immunogenic and prioritized for further testing. Allergenicity analysis was performed using AllerTOP v.2.0. Peptides classified as non-allergenic were selected to minimize the risk of adverse immune reactions in potential vaccine applications. The results of these five peptides have the potential to be antigens and of the five peptides, only 3 peptides have non-allergenic allergenic abilities (Table 2).

Table 2. Antigenicity and Allergenicity Test Results of Candidate Peptides

Peptide	Antigenicity	Allergenicity
NITTDSRCPTQGEAVLPEEQDQN	0.7551 probable antigen	non-allergen
GDQHQVGNETQGVTAEITPQASI	0.5393 probable antigen	non-allergen
AGPISQHNHRPGYHTQTAGPW	0.7841 probable antigen	allergen
VEPGKNPKNFQTMPGTFQTTTGEI	0.6698 probable antigen	non-allergen
VVTKKEEPVNIEAEPPFGE	1.2189 probable antigen	allergen

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Toxicity, *Instability*, and Immunogenicity Test

The three peptide series are expected to be non-toxic to the human body based on the experiments that have been conducted. To get peptides that are not harmful when given to the human body, toxicity testing is crucial at the vaccine prediction stage[12]. Peptides can only be chosen for additional use if their toxicity values are negative [13]. The hydrophilicity value was also discovered by this test. Based on table 2, the results obtained are that the three peptides obtained non-toxic results. Which means that further testing can be carried out. Furthermore, a vaccine design stability test was carried out using protparam. The Protparam test is used to analyze the physicochemical properties of peptides, (molecular weight, half-life, sequence length, aliphatic index, instability index, theoretical pI, and average hydropathic)[14]. Obtaining the results of 2 peptides (GDQHQVGNETQGVTAEITPQASI and VEPGKNPKNFQTMPGTFQTTTGEI) which have a stable index while 1 peptide (NITTDSRCPTQGEAVLPEEQDQN) has an unstable index. Then the Immunogenicity test obtained the results, namely the peptide NITTDSRCPTQGEAVLPEEQDQN obtained an immunogenicity value of 0.04846, peptide GDQHQVGNETQGVTAEITPQASI obtained an immunogenicity value of 0.20207, peptide VEPGKNPKNFQTMPGTFQTTTGEI obtained an immunogenicity value of -0.28446

Table 3. Toxicity, Instability, and Immunogenicity Test Results of Candidate Peptides

Peptide	Toxicity	Instability Index	Immunogenicity
NITTDSRCPTQGEAVLPEEQDQN	Non-Toxin	unstable	0.04846
GDQHQVGNETQGVTAEITPQASI	Non-Toxin	stable	0.20207
VEPGKNPKNFQTMPGTFQTTTGEI	Non-Toxin	stable	-0.28446

Structural Validation

ElliPro was used to validate the predicted epitopes' structural protrusion, solvent accessibility, and flexibility. These characteristics are essential for ensuring the selected epitopes are accessible for immune system recognition. Linear epitope prediction of B cells using the Kolaskar & 9 Tongaonkar Antigenicity method produces 8 predicted peptides in Figure 1. This method uses experimental data of antigenic determinants and physicochemical properties of amino acids for epitope prediction. This method was developed to predict antigenic determinants and has an accuracy of 75% [15]. B cell epitope prediction using the Kolaskar & Tongaonkar Antigenicity method. Average value: 0.004, Minimum: 0.002, Maximum: 2.812. Based on Figure 2. The selection of residue images was chosen based on the highest residue numbers, namely residue numbers 46 and 47, which can be seen in Table 3.

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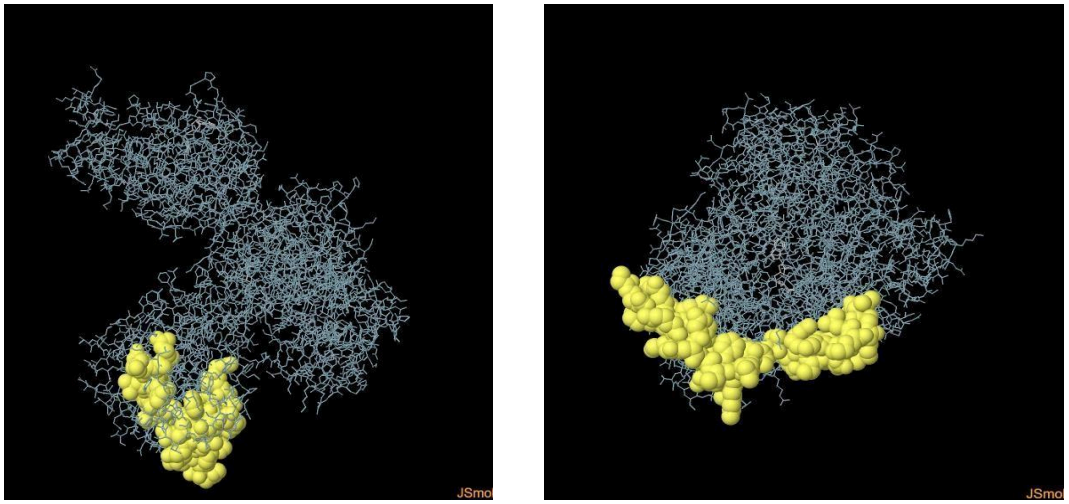

(A) (B)

Figure 2 Result of ElliPro analysis - Epitope prediction based upon structural protrusion which has the highest number of residues

Table 4. Epitope linear prediction ElliPro: Antibody Epitope Prediction

Chain	Start	End	Peptida	Number of Residu	Score
A	3259	3305	RRDLRLASNAICSAVPVHWVPTSRTTWSIHAHHQWMTTEDMLTVWNR	47	0.713
A	2496	2541	GETLGEKWKKKLNQLTRKEFDLYKKSGITEVDRTEAKEGLKRGEIT	46	0.831

Moreover, the Swiss model is used in 3D modeling. An automated service for 3D protein structural comparison is called Swiss-Model. Since 1993, Swiss-Model has led the way in automated modeling and is currently the most popular free web-based tool for automated modeling. In a web-based workspace, Swiss-Model combines the databases and software needed for protein structure modeling. The target protein's amino acid sequence is supplied as input data in order to create a three-dimensional model. The server does all of the template selection, alignment analysis, and model development automatically. Figure 3 shows the outcomes of 3D modeling.

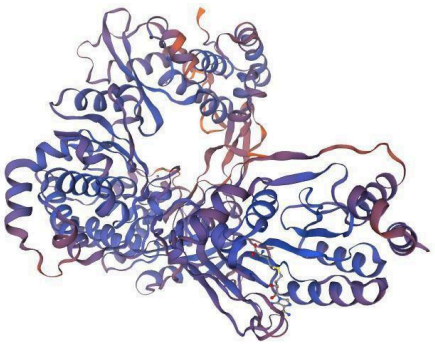

Figure 3. 3D Modelling Result of Swiss Model

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Discussion

The bioinformatics approach used in this study successfully identified peptide candidates for a potential dengue virus type 3 vaccine based on polyprotein sequences. Two peptides, GDQHQVGNETQGVTAEITPQASI and VEPGKNPKNFQTMPGTFQTTTGEI, were selected due to their favorable characteristics in terms of antigenicity, non-allergenicity, non-toxicity, and stability. This study reinforces the importance of in silico methods in narrowing down epitope candidates before advancing to wet-lab testing, which is resource-intensive and time-consuming[16]. These methods are particularly useful for rapidly evolving viruses like DENV, where the antigenic landscape may shift over time and across geographic regions.

Interestingly, while VEPGKNPKNFQTMPGTFQTTTGEI showed strong performance across most parameters, it had a negative immunogenicity score (-0.28446), suggesting potential limitations in eliciting an effective immune response. This highlights that peptide vaccine design must balance physicochemical suitability with true immunogenic potential[17].

The peptide GDQHQVGNETQGVTAEITPQASI, with a stable structure and positive immunogenicity value (0.20207), may serve as a more promising candidate. However, its antigenicity score (0.5393) was relatively lower than other peptides, indicating a trade-off between different properties in epitope selection. Comparison with previous studies shows similar reliance on IEDB, VaxiJen, and ProtParam tools [9], though this study is one of the few that specifically targets DENV3-5_S_162, a relatively underexplored isolate. This novelty enhances the scientific relevance of the findings and opens opportunities for serotype-specific vaccine development, particularly in Southeast Asia where DENV3 is dominant.

Both identified peptides are located within regions of the polyprotein that likely correspond to structural envelope (E) or non-structural (NS) proteins, which are known to be key immunogenic components in dengue virus pathogenesis. The E protein plays a vital role in viral attachment, membrane fusion, and entry into host cells. Peptides derived from E protein are thus more likely to elicit neutralizing antibody responses[18].

In particular, GDQHQVGNETQGVTAEITPQASI is predicted to lie in a region of the envelope protein that may be exposed on the virion surface. This enhances its accessibility to B-cell receptors and promotes humoral immune recognition. Its stable structure and positive immunogenicity score further support this possibility[19].

On the other hand, VEPGKNPKNFQTMPGTFQTTTGEI may be derived from a segment of the non-structural NS1 or NS3 protein, which play important roles in viral replication and immune evasion. NS1 is known to be secreted and can interfere with complement activation and endothelial barrier function. While it may be immunogenic, responses against NS1 might not be as neutralizing as those against the E protein, which may explain its lower immunogenicity score despite good physicochemical parameters[20].

Mechanistically, recognition of these peptide epitopes by antigen-presenting cells (APCs) through major histocompatibility complex (MHC) class II presentation could activate CD4+ T-helper cells, which in turn enhance B-cell maturation and antibody production. The high antigenicity and non-allergenicity suggest that the peptides can effectively be processed without inducing hypersensitivity responses[21].

Furthermore, the stability index indicates that these peptides are less likely to undergo rapid degradation in vivo, which is critical for maintaining their structural integrity during antigen presentation. This is especially important for peptide vaccines, which are otherwise prone to proteolytic cleavage. Thus, the selection of peptides with structural stability and surface accessibility, coupled with antigenic and immunogenic

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potential, is highly aligned with the molecular criteria required for an effective peptide vaccine against flaviviruses like DENV.

Moreover, future in vitro and in vivo validation could explore whether these peptides induce neutralizing antibodies, cytokine responses (e.g., IL-2, IFN-γ), or cytotoxic T lymphocyte (CTL) activation. Such studies would clarify the downstream molecular cascades and immune pathways engaged by these epitopes, further validating their use as subunit vaccine candidates.

In summary, this study presents a comprehensive pipeline for peptide-based vaccine prediction against DENV-3. The results serve as a valuable reference for targeted vaccine development and highlight the strengths and limitations of in silico approaches in the preclinical stage. Integrating biomolecular insights strengthens the rationale for selecting GDQHQVGNETQGVTAEITPQASI and VEPGKNPKNFQTMPGTFQTTTGEI as promising targets for next-phase experimental validation.

Conclusion

From the analysis conducted, two peptides, GDQHQVGNETQGVTAEITPQASI and VEPGKNPKNFQTMPGTFQTTTGEI, were identified as potential vaccine candidates for Dengue virus type 3 isolate DENV3-5_S_162. These peptides successfully passed multiple tests, including antigenicity, allergenicity, toxicity, instability index, and immunogenicity assessments. The results indicate that these peptides possess the necessary properties to be further developed as peptide-based vaccines for Dengue virus type 3. This study provides a foundational reference for future research and development in the field of Dengue vaccine production.

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Conflict of Interest

The authors declare no conflict of interest.

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All authors have read and agreed to the published version of the manuscript.

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