JTL 13 (2) December 2024
Page: 96-105

Contents list available at Jurnal Teknologi Laboratorium

JURNAL TEKNOLOGI LABORATORIUM

Journal Homepage: www.teknolabjournal.com
ISSN 2580-0191(Online) I ISSN 2338 – 5634(Print)

Original Research

OPEN ACCESS

Epitope-Driven Vaccine Development for Zika Virus: A Bioinformatics Approach

Riska Ayu Sutriyansyah[1], Muhamad Hilmi Ihsanul Iman[1], Cahya Ajeng Valenta Tresna Sulung[1], Nelly Indira Kusuma Wardani[1], Arif Nur Muhammad Ansori[2,3,4,5]*

[1] Study Program of Biology, Faculty of Mathematics and Natural Sciences, Surabaya State University, Surabaya, Indonesia
[2] Postgraduate School, Universitas Airlangga, Surabaya, Indonesia
[3] Uttaranchal Institute of Pharmaceutical Sciences, Uttaranchal University, Dehradun, India
[4] Virtual Research Center for Bioinformatics and Biotechnology, Surabaya, Indonesia
[5] Division of Research and Development, Jalan Tengah, Surabaya, Indonesia

Abstract: Zika virus (ZIKV) has become a global concern in 2015-2016, which can infect adults and develop fetuses, has become a global concern. ZIKV is a member of the Flaviviridae family, which can spread through Aedes mosquitoes, sexual intercourse from mother to fetus, and blood transfusions. The genetic material is a single-stranded RNA with positive polarity. Conventional vaccine development requires a long time and significant resources; therefore, the bioinformatics approach is an efficient alternative for identifying B cell epitopes as vaccine candidates. This study used bioinformatics or *In silico* methods to identify B cell epitopes with the immune epitope database (IEDB) web server. This study showed that the peptide sequence of "EWFHDIPLPWHAGADTGTPHWNNKEA" (peptide 6E) in the envelope protein E of ZIKV is a potential vaccine candidate. This peptide is predicted to exhibit high antigenicity, non-allergenicity, and non-toxicity. This study concludes that peptide 6E is a promising vaccine candidate. Further studies are needed *in vitro* and *in vivo* to confirm that it can be used as a potential ZIKV vaccine candidate and applied in the future.
Keywords: Epitope; Medicine; Peptide; Vaccine; Zika Virus

INTRODUCTION

Zika virus (ZIKV) is a member of the Flavivirus genus of the Flaviviridae family and is closely related to dengue (DENV), West Nile (WNV), Japanese Encephalitis (JEV), and Yellow Fever (YFV) viruses. ZIKV was first isolated from rhesus monkeys in Uganda, in 1947. Transmission mainly occurs through *Aedes* mosquitoes, particularly *Ae. aegypti* and *Ae. albopictus*, with humans as the primary host. ZIKV can be transmitted through sexual intercourse, vertical transmission from mother to fetus, and blood transfusion[1]. This statement was further strengthened by research by Pattnaik (2020), who stated that apart from mosquito bites, ZIKV can also spread through sexual intercourse, from mother to fetus, and through blood transfusions[2]. The ZIKV genome consists of positive single-stranded RNA that is translated into a single polyprotein and processed into three structural proteins (C, prM, and E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). The E protein is the main target of

Corresponding author.
E-mail address: ansori.anm@gmail.com (Arif Nur Muhammad Ansori)
DOI: 10.29238/teknolabjournal.v13i2.518
Received 21 July 2024; Received in revised form 27 September 2024 Accepted 21 October 2024

the neutralizing antibody response, with the most potent epitope located in domain III of the E protein. Effective antibodies bind strongly to and block areas of the E protein, which are crucial for the entry of the virus into host cells[3].

The Zika virus (ZIKV) has become a global concern since a significant outbreak in the Americas in 2015–2016, which revealed the potentially severe impact of this infection, especially on the developing fetus[1]. Although ZIKV cases have decreased significantly since then, the threat of future outbreaks remains, prompting ongoing efforts to develop effective and safe vaccines. Conventional vaccine development often requires a considerable amount of time and resources. Several researchers have previously attempted to design vaccines and identify the best candidates for T- and B-cell epitopes using immunoinformatics approaches[4]. The adaptive immune reaction against ZIKV is mediated by B cells, which produce antibodies in humoral immunity, and T cells, which produce antibodies in cellular immunity. The viral proteins E, prM, and NS1 are the main targets of the neutralizing antibody response caused by ZIKV in humoral immunity and, are very important for protection against viral infections. Several isolated human monoclonal antibodies (mAbs) have shown high specificity against all ZIKV strains and strong protective activity both *in vitro* and *in vivo*[5]. Bioinformatic approaches offer a more efficient and faster alternative. Bioinformatics enables the identification of potential B-cell epitopes as vaccine candidates through the analysis of genetic and protein data from ZIKV. B-cell epitopes are antigens that are recognized by antibodies to stimulate a specific and protective immune response against viruses. The E protein exhibits strong antigenicity, which means it can effectively stimulate both B cell and T cell responses. Some studies have indicated that targeting specific epitopes with in the E protein can elicit strong immune responses, so that making it a viable target for both subunit and epitope-based vaccines[6].

Vaccine development can be accelerated using immunoinformatics and computational methods, which are also cost-efficient ways to develop simulations and calculations in drug design. Epitope-based vaccine design has emerged as a promising approach in modern vaccine development, offering advantages in terms of selectivity, safety, and ability to stimulate focused immune responses[3]. This method involves the identification and selection of highly immunogenic viral epitopes through in-depth analysis of viral protein sequences. Protein- or peptide-based vaccines allow for more precise targeting of specific epitope regions in viral antigens, thereby inducing a strong and specific immune response. *In silico* approaches and modern bioinformatics techniques have accelerated the process of identifying potential epitopes, enabling the prediction of B- and T-cell epitopes with greater accuracy[7]. In addition, the use of bioinformatic methods in the design of B-cell epitope-based vaccines can also accelerate the development of ZIKV vaccines. Through *in silico* analysis, epitopes with the potential to stimulate a strong immune response can be identified with high precision.

Bioinformatics approaches in vaccine design have undergone significant development, offering greater time and cost efficiency, as well as flexibility in responding to the rapid evolution of pathogens such as ZIKV[8]. Recent machine learning and artificial intelligence algorithms have improved the accuracy of epitope and antigenicity predictions, enabling the rapid adaptation of vaccine designs to emerging virus variants[9]. Through this article, the author will explore bioinformatics methods in the design of the best ZIKV vaccine candidate in terms of the position of the B cell epitope, highlighting the advantages, challenges, and prospects of this approach in efforts to overcome ZIKV outbreaks and improve preparedness for similar infectious diseases in the future.

MATERIAL AND METHOD

Protein Preparation, Modelling, and Validation

The ZIKV protein used in this study was envelope protein E, which was obtained from the NCBI database (https://www.ncbi.nlm.nih.gov/) with accession number XBA21084.1, and the sample was downloaded in the FASTA format. The 3D structure of the ZIKV E protein was obtained from the results of homology modelling performed using the SWISS-MODEL web server (https://swissmodel.expasy.org/). This method aligns the query sequence to determine the 3D structure of the target protein[10,11]. The 3D model was validated using a Ramachandran plot with a score threshold of 90%[12,13].

B-cell Epitope Mapping

B cell epitope mapping of the ZIKV E protein was predicted using The Immune Epitope Database (IEDB) web server (http://tools.iedb.org/bcell/) with the BepiPred-2.0 tool (https://services. healthtech. dtu. dk /service. php?BepiPred-2.0). BepiPred predicts the epitope region of a target protein or peptide candidate that can be identified based on the specific epitope position where the sequence will be input to The Immune Epitope Database (IEDB) with a threshold of 0.5[14,15].

Identification Antigenicity and Allergenicity

Identification of peptide antigenicity using the VaxiJen v2.0, web server (https://www.ddgpharmfac.net/vaxijen/VaxiJen/VaxiJen.html), to trigger an immune response, which is predicted to have a score greater than the threshold value of 0.4[16]. Allergenicity prediction aims to determine the level of potential allergens and to ensure that the peptide is not an allergen as a vaccine candidate. This prediction uses AllerTOP v2.0 (https://www.ddgpharmfac.net/AllerTOP/)[17].

Prediction of Toxicity Level and Physicochemical Properties

The toxicity level of peptide candidate vaccines must be determined using ToxinPred (https://www.crdd.osdd.net/raghava/toxinpred/) to determine whether the peptide candidate is not toxic with a threshold value of 0.1[18]. The physicochemical properties of the candidate peptides were predicted using the ProtParam web server (https://www.web.expasy.org/cgi-bin/protparam/protparam) to identify the average hydrophobicity score (GRAVY), aliphatic index, instability, molecular weight, and theoretical pI[19].

Selection of Vaccine Candidate Peptides and 3D Visualization of Epitopes

The selection of peptides as vaccine candidates can be seen from sequences that have antigenicity, non-allergenicity and non-toxicity results or can be seen from the results of cell epitope potential graphs from The Immune Epitope Database (IEDB). The best peptide was visualized in 3D to determine the target position or region on the ZIKV E protein, which allowed it to be recognized or act as a B cell epitope using PyMol v.2.5.5 software through an academic license.

RESULTS AND DISCUSSION

Protein Modelling and Validation

In this study, ZIKV Envelope Protein E was used as a vaccine design target and the 3D structure was modeled using SWISS-MODEL, which consists of β-sheets, α-helices, and coil structures. The Ramachandran score plot shows no Bad Bonds (Bad Bonds 0/2311), 91.53% favorite bonds, 0.34% outliers, and GMEAN and QMEAN values of 0.81 and -3.74 respectively. The protein model identification score was 100%. Local quality estimation is related to the estimation of each model residual (x-axis) and the expected similarity value to the original structure (y-axis).

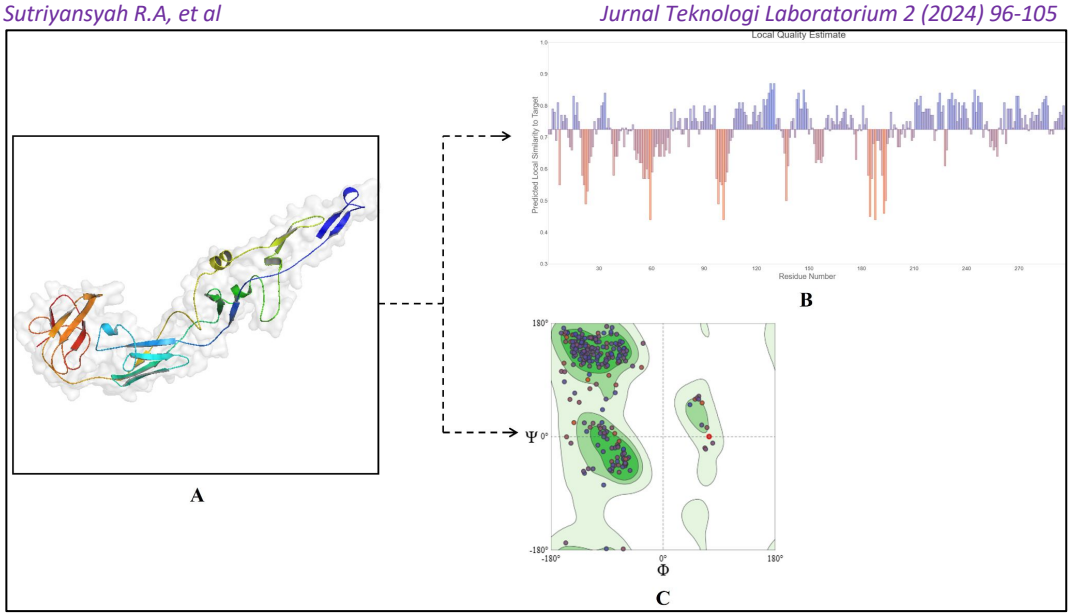

Figure 1. ZIKV Protein Visualization. (A) 3D Structure of ZIKV Envelope Protein E; (B) Graph of Estimated Local Quality of Envelope Protein E ZIKV; (C) Ramachandran Plot Results.

Fluctuating graph (Fig. 1B) shows similarity scores lower than 0.5 for some of the residues that form the model with the template. The weak bonds in the Ramachandran plot are weakly related to chemical interactions, such as van der Waals bonds that weakly affect protein structure, compared to the results of hydrogen bond interactions (Fig. 1C). However, the ZIKV E protein model was considered valid because it has a similarity value of 100%.

B cell epitope mapping, antigenicity, allergenicity, toxicity, prediction of physicochemical properties

B cells from the ZIKV E protein predicted using EIDB (The Immune Epitope Database) with the peptide naming ending in "E" obtained 11 epitopes, namely the longest 26-mer found at position 122–147, and the shortest 2-mer found at positions 80–81 and 256–257 (Table 1). To determine a good vaccine candidate, it is necessary to predict antigenicity, allergenicity, and toxicity. Of the 11 peptides in the ZIKV E protein predicted using VaxiJen, AllerTOP Server, and ToxinPred, peptide 6E was obtained (Table 2)., which was used for further analysis because it is thought to initiate the formation of a B cell immune response[20].

Table 1. Results of B-Cell Epitope Mapping.

Peptide	Posisi	Length	Peptide Sequence
1E	6–9	4	GWGN
2E	33–39	7	GKSIQPE
3E	52–69	18	SQHSGMIVNDTGHETDEN
4E	80–81	2	PR
5E	100–103	4	TGLD
6E	122–147	26	EWFHDIPLPWHAGADTGTPHWNNKEA
7E	153–156	4	DAHA
8E	219–228	10	TFTKIPAETL
9E	241–243	3	TDG
10E	256–257	2	QT
11E	274–277	4	STEN

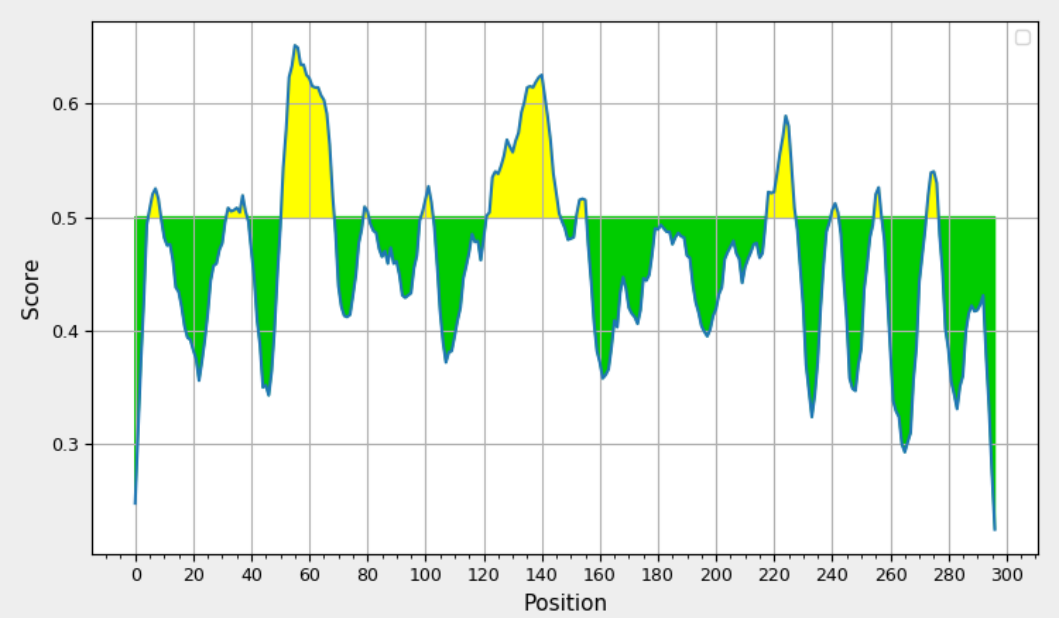

Figure 2. B-Cell Epitope Potential Graph.

Various methods have been used to analyze B-cell epitopes, such as the Kolaskar and Tangaonkar method, which is used to predict specific areas of proteins that bind to B cell receptors. These areas must be on the surface and be immunogenic[21]. The results of the B-cell mapping are displayed in a graphical form (Fig. 2) The yellow area shows sequences that have potential B-cell epitopes, while the green color shows sequences that do not have potential B-cell epitopes[15].

Table 2. Antigenicity, Allergenicity, and Toxicity Prediction Results.

Peptide	Antigenicity	Allergenicity	Toxicity
1E	-	Non-allergen	Non-toxin
2E	Antigen	Allergen	Non-toxin
3E	Non-antigen	Allergen	Non-toxin
4E	-	Allergen	Non-toxin
5E	-	Allergen	Non-toxin
6E	**Antigen**	**Non-allergen**	**Non-toxin**
7E	-	Non-allergen	Non-toxin
8E	Non-antigen	Non-allergen	Non-toxin
9E	-	Allergen	Non-toxin
10E	-	Allergen	Non-toxin
11E	-	Allergen	Non-toxin

Table 3. Prediction Results of Physicochemical Properties

Peptide	Theoretical pI	MolecularWeight (Da)	GRAVY	Index	
				Aliphatic	Instability
6E	5.21 (antigen)	3027.26	-1.065 (non-allergen)	41.54	6E

The physicochemical properties of the peptide 6G were predicted using ProtParam (Table 3). Candidate vaccines have a molecular weight of 36 kDa. Apart from that, a GRAVY score is also needed, namely between -0.14 – -0.45, to allow natural hydrophilic interactions to form in the vaccine, but peptide 6E does

not meet this requirement. The aliphatic index score for peptide 6E was also low, between 64.13 and 80.42, indicating less stability at some temperatures. However, the instability index score for peptide 6E was low (<40) with a value of 12.04. This allows for stability when built and triggers the initiation of an immunogenic reaction[19].

Selection of vaccine candidate peptides and 3D visualization of epitopes

Epitope position selection was carried out on peptide 6E, namely the sequence "EWFHDIPLPWHAGADTGTPHWNNKEA, " at positions 122–147. Sequence display was perfomed by selecting or blocking the target position sequence using PyMol software.

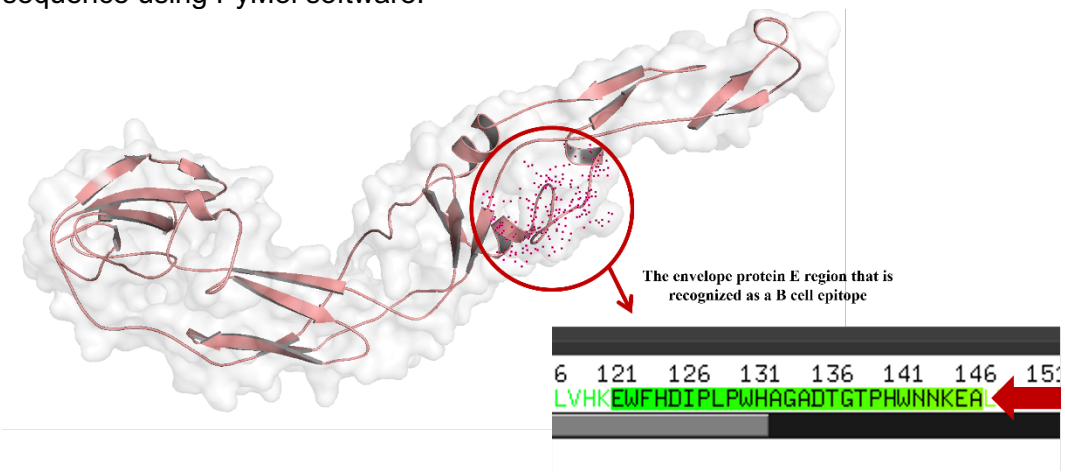

Figure 3. 3D Visualization of B-Cell Epitope Target Positions

3D visualization of the ZIKV E envelope protein region is needed to determine the position that allows it to be recognized or act as a B-cell epitope, as shown in (Fig. 3) above.

Bioinformatics has enabled rapid progress in the field of vaccinology, and vaccines developed using modern techniques are safer, more effective, and more cost-effective than traditional vaccines[20]. However, obtaining an appropriate immunological response requires a deep understanding of the pathogen, particularly genome analysis and epitope prediction[22]. The great potential of epitopes for vaccine development, disease prevention, diagnosis, and treatment has attracted significant research interest[23]. Using state-of-the-art technology, specific epitopes that can replace all pathogens in vaccines have been isolated. However, not all epitopes exhibit the same ability to produce antibodies[24].

In silico research on vaccine design based on epitopes has been carried out for various types of viruses, such as Zika virus (ZIKV)[22], and Dengue Virus (DENV)[25]. In this study, we aimed to create a vaccine based on ZIKV E protein of ZIKV based on ZIKV data from the NCBI reference sequence with accession number XBA21084.1, which has a genome size of 297 amino acids (aa) as a vaccine design target. The presented vaccine candidate showed significant potential through *in silico* analysis, but further *in vitro* and *in vivo* investigations are needed to confirm its effectiveness.

Generally, epitope reactions with antibodies occur on the protein surface; therefore, the amino acids that make up B-cell epitopes tend to be hydrophilic. Therefore, in this study, the hydrophilicity of amino acid residues was used as a criterion for predicting B cell epitopes. Most epitopes recognized by antibodies are discontinuous epitopes, namely in the form of a sequence of amino acids that are not sequential or discontinuous in their primary structure but are close together. Each has a folded three-dimensional (3D) structure[26,27]. However, the ZIKV E protein model produced through SWISS-MODEL was considered valid because it had a similarity value of 100%.

Results from B cell epitopes using The Immune Epitope Database (IEDB) to predict antigenicity, allergenicity, and toxicity on the ZKIV Virus E protein produced 11 peptides on the ZIKV E protein, which were predicted via VaxiJen, AllerTOP Server, and ToxinPred, and obtained peptide 6E, which met the requirements for vaccine candidates because it contains antigens, non-allergens, and non-toxicity. Peptide 6E contained a high antigen level (5.21). Antigenicity is one of the properties of antigens that triggers a B cell response to produce specific antibodies, which refers to immunogenicity[28]. The peptide in 6E has a high antigenicity value because the recommended vaccine has a minimum antigenicity of 0.5558, which allows the body to recognize it as an antigen[29].

Vaccine designs with B cell epitopes have been widely developed because B cells are part of the proteins that trigger the immune system or bind to antibodies. Vaccine production must meet safe levels by considering the non-allergenic and non-toxic design of vaccines. One peptide that meets this requirement is 6E, which is targeted for vaccine design because it is non-allergenic and non-toxic. The non-allergenic results obtained indicate that they do not cause allergic reactions in vaccine candidates because allergies can cause reactions that are dangerous to the owing to excessive activity that interferes with immunopathology[30]. In addition, vaccine design must be non-toxic because toxicity itself is a compound that is capable of causing toxic harmful effects on organisms, causing an imbalance in the vaccine administered to the candidate, which triggers the presence of toxins and excessively disrupts the body's working mechanisms[31].

Designing Zika virus (ZIKV) vaccine candidates based on B-cell epitopes has been a promising vaccine development approach because of its ability to target specific viral components that can trigger a strong immune response. B cell epitopes are part of the antigen that is recognized by antibodies, and by designing vaccines that target these epitopes, we can increase the effectiveness of the vaccine in protecting against ZIKV infection. This approach involves identifying and mapping the B-cell epitope of the ZIKV envelope protein (E), which is the main target of neutralizing antibodies. Recent studies have used bioinformatics to predict the potential B-cell epitopes of the ZIKV E protein. For example, Gupta (2023) used computational modelling techniques to identify B-cell epitopes that can trigger a protective immune response[32].

Additionally, epitope-based vaccines can be tested in various *in vitro* and *in vivo* models to evaluate their immunogenicity and protective efficacy. Zhang (2020) showed that a vaccine candidate based on B-cell epitopes can stimulate the production of antibodies capable of effectively neutralizing ZIKV in a mouse model[33]. These results demonstrate great potential for the development of an effective anti-ZIKV vaccine using a B-cell epitope-based approach. Therefore, the design of a B-cell epitope-based ZIKV vaccine offers an innovative and specific strategy to protect the public from ZIKV infection.

CONCLUSION

From the results of this study, the "EWFHDIPLPWHAGADTGTPHWNNKEA" peptide (peptide 6E) is a good candidate for the ZIKV vaccine because it has high antigenicity, low toxicity, and does not trigger an autoimmune response. In addition, it can increase the immune response of B cells through activation and differentiation into plasma cells, formation of memory cells, and increase IgM or IgG antibody titers for virus neutralization. However, this needs to be further verified through *in vivo* and *in vitro* experiments to ensure the effectiveness and safety of peptide 6E as a vaccine candidate.

AUTHORS' CONTRIBUTIONS

M. Hilmi Ihsanul Iman prepared the samples; Cahya Ajeng designed the research protocols; all authors executed the research protocols; Riska Ayu Sutriyansyah and Nelly Indira Kusuma Wardani wrote the manuscript. Arif Nur Muhammad Ansori reviewed and supervised the study. All the authors have read and approved the final manuscript.

ACKNOWLEDGEMENT

We thank Jalan Tengah, Indonesia (www.jalantengah.site) for editing the manuscript.

FUNDING INFORMATION

None.

DATA AVAILABILITY STATEMENT

The data utilized in this investigation are available from the corresponding author up reasonable request.

DISCLOSURE STATEMENT

The data are the results of the author's research and have never been published in other journals.

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