ISSN 2460-1365 **Bioeksperimen,** Volume 9 No. 2 (September 2023)

Expression and Activation of Toll-Like Receptor 9 in Dengue Virus Infection: A Scoping Review

Safari Wahyu Jatmiko[1,2)]*, Riandini Aisyah[1,3)]

[1)]Department of Biomedical Sciences, Faculty of Medicine, Universitas Muhammadiyah Surakarta, Sukoharjo, Central Java 57169, Indonesia

[2)]Clinical Pathology Laboratory, Faculty of Medicine, Universitas Muhammadiyah Surakarta, Sukoharjo, Central Java 57169, Indonesia

[3)]Molecular Biology Laboratory, Faculty of Medicine, Universitas Muhammadiyah Surakarta, Sukoharjo, Central Java 57169, Indonesia

*Corresponding e-mail: safari.wahyu@ums.ac.id

Received: 10 September 2023, Revised: 28 October 2023, Available Online: 10 November 2023

Abstract *– Toll-like receptors (TLR) are essential to pathogen recognition to activate innate immunity. TLR2, 3, 4, 6, 7, 8, and 9 are involved in immunity to dengue virus. The involvement of TLR9, known to recognize CpG DNA, in dengue virus (DVI) infection, an RNA virus, is unusual. We hypothesize that there is an elevated expression and activation of TLR9 through specific mechanisms in DVI. A scoping review was carried out to collect articles that have been written regarding this topic. Article searches were carried out on the PubMed (Medline), Proquest, EBSCO, and Google Scholar databases with inclusion criteria: articles in the form of research results using human subjects or human cells, conducted from 2013 to 2023, in English, and can be accessed in full -text. Gray articles, books, and review articles were excluded from the study. The literature search results found 821 articles, with eight articles meeting the criteria. Based on mapping, synthesis, and analysis of the articles obtained, it was found that there was increased expression and activation of TLR9 in the DVI due to the release of mtDNA.*

Keywords: *Dengue Virus Infection, CpG DNA, expression, TLR-9, mtDNA*

INTRODUCTION

The dengue virus (DENV) is the etiological agent responsible for Dengue virus infection (DVI). The dengue virus comprises four distinct types, referred explicitly to as DENV1 to DENV4 (Guzman & Harris, 2015). The four types of DENV can induce DVI from moderate to severe symptoms (Malavige & Ogg, 2017) due to the immunological response of patients infected with DENV might differ (Sprokholt et al., 2018).

The immunological response to Dengue virus (DENV) is initiated upon the recognition of DENV by the immune system. Immunocytes hold pathogen recognition receptors (PRRs) such as RIG-I-like receptors, NOD-like receptors, cyclic GMP-AMP synthase, and Toll-like receptors (TLRs) that recognize pathogen-associated molecular patterns (PAMPs) of DENV. These PAMPs involve RNA genetic material, structural proteins, and non-structural proteins. (Fernandes-Santos & de Azeredo, 2022; Sprokholt et al., 2018).

The Toll-like receptors (TLRs) that exhibit recognition of DENV encompass TLR2, TLR3, TLR4, TLR6, TLR7, and TLR8 (Chao et al., 2019; Chen et al., 2015; Kayesh et al., 2021; Modhiran et al., 2017). The recognition of NS-1 is attributed to Toll-like receptors 2, 4, and 6 (Chao et al., 2019; Chen et al., 2015; Modhiran et al., 2017), TLR3, TLR7, and TLR8 are capable of identifying the genetic material of DENV in the RNA form. (Kayesh et al., 2021). The binding between TLRs and PAMPs from DENV triggers the activation of intracellular signaling pathways, both MyD88-dependent and TRIF-dependent (Balka & De Nardo, 2019; El-zayat et al., 2019; Gay, 2019; Ullah et al., 2016). The activation of TRAF6 is initiated by the generation of Myddosome, which is triggered by intracellular signals

originating from MyD88. The activation of TAK1 by TRAF6 subsequently induces the activation of MAPK and IKK. Both activation routes initiate the production of proinflammatory cytokines (Balka & De Nardo, 2019; Ullah et al., 2016). TRIF-dependent intracellular signaling pathways trigger the activation of IKK and TRAF3. IKK stimulates the expression of proinflammatory cytokines, while TRAF3 activates IRF3 and 7 to stimulate the formation of type 1 interferon (IFN) (Ullah et al., 2016). Proinflammatory cytokines and type 1 IFN play a role in antiviral mechanisms and are responsible for the emergence of severe DVI (Kuczera et al., 2018; Wang et al., 2018).

Torres et al. (2013) findings indicated a rise in TLR9 expression on myeloid dendritic cells (mDC) and alterations in the generation of IFN I response in mDC infected with DENV. This observation suggests that Toll-like receptor 9 (TLR9) has a response to DVI stimuli (Kim et al., 2013). TLR9 is an endogenous TLR that specifically recognizes genetic material in the form of CpG-DNA. (Torres et al., 2013), while DENV is classified as an RNA virus (Cipitelli et al., 2019). This issue pertains to the underlying mechanisms through which DENV, an RNA virus, can trigger the activation of TLR9. Based on a preliminary search of the PubMed and Google Scholar databases, no scoping reviews or other literature reviews have been identified that particularly investigate the activation of TLR9 in DVI. Thus, this scoping review aims to address the issue of TLR9 activation in DVIs.

MATERIALS AND METHODS

A scoping review was conducted to determine research gaps with the methodology that was proposed by Arksey & O'Malley (2005). The scoping review procedure consists of five distinct stages: formulation of research questions, development of study design and literature search strategy, screening of relevant material, mapping of data, compilation, summarization, and reporting of findings.

1. Research Questions

1. Is there an observed increase in the expression of TLR9 in the DVI?
2. Does TLR9 activation develop in the DVI?
3. How is the activation mechanism of TLR9 by DENV, an RNA virus?

2. Study design and literature search approach

Scoping reviews are conducted utilizing scientific articles collected from electronic databases. The research literature was systematically reviewed utilizing various databases, including MEDLINE (PubMed), EBSCO, Proquest, and Google Scholar. The search was undertaken using specific keywords (dengue or "dengue virus" or "dengue virus infection") and ("Toll-like Receptor 9" or TLR9 or TLR-9 or "TLR 9"). The inclusion criteria applied include studies with human samples or human cells, written in English, available in full text, and conducted between 2013 to 2023. The study omitted review articles, gray literature, and books from its analysis.

3. Articles Screening

The articles sourced from electronic databases underwent a process of duplicate screening. The screening process for articles involves evaluating their relevance to the restriction criteria, which occurs after the initial screening for duplication. The screening process involves evaluating the appropriateness of the abstract regarding the research topic. The textual content and bibliography of each relevant article is thoroughly examined to ascertain its

appropriateness for the given subject matter. Two reviewers analyzed of articles that had the potential to serve as reference sources. The articles that received agreement from two reviewers were selected for examination. The outcome of discrepancies between the first and second reviewers is decided by attaining an agreement among them.

4. Data Mapping

All articles obtained were mapped, referring to Pawson (2002). Mapping process involves the construction of a comprehensive table that encompasses various vital elements, including the author's identity, publication year, research location, target population, study design, measured variables, and measurement outcomes.

5. Organizing, Summarizing, and Reporting Results

The available literature is described descriptively as a way to map general and specialized information. In order to address the research inquiries, articles are classified into three distinct categories: TLR9 expression, TLR9 activation, and the processes underlying TLR9 activation by DENV.

RESULTS AND DISCUSSION

1. Result

A total of 821 articles were identified based on the article search results. A total of eight articles were included for discussion. The steps of article screening are illustrated in Figure 1. Descriptive information regarding the literature is presented in Table 1, while the results of the literature mapping are presented in Table 2.

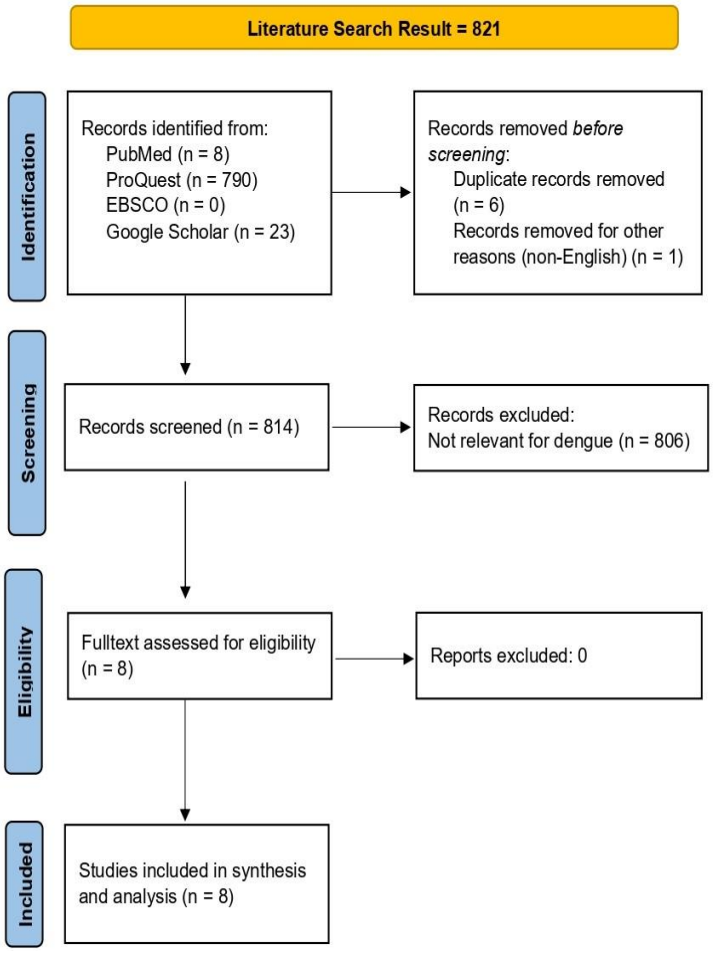

Figure 1. The steps of article screening

Table. 1. Characteristics of included studies

Characteristics	N (%)
Total articles reviewed	8
Research area	
South America	3 (37.5)
East Asia	2 (25)
South Asia	3 (37.5)
Study design	
Experimental	5 (62.5)
Analytical observational	3 (37.5)
Degree of DVI of research subjects (people)	
Mild	159
Severe	58
Healthy Individu	121
Types of viruses used in studies	
DENV1	2 (16.7)
DENV2	5 (41.7)
DENV3	3 (25)
DENV4	2 (16.7)
Type of cells being investigated	
Monocyte	1 (14.3)
Monocyte-derived macrophage (MDM)	2 (28.6)
Dendritic cell (DC)	2 (28.6)
Monocyte-derived dendritic cells (Mo-DC)	1 (14.3)
Corneal tissue	1 (14.3)
The primary findings of the studies	
TLR9 expression	4 (36.4)
TLR9 activation	5 (45.5)
Activation mechanism of TLR9	2 (18.2)

Table 2. Literature Mapping

Author (year)	Geographical	Targets	Study design	Parameters	Outcome
Castillo et al., (2021)	Medellı´n, Colombia	MDM	Experimental	TLR9 Expression	VitD decreases TLR9 expression in DVI
Castillo & Urcuqui-Inchima, (2023)	Medellı´n, Colombia	MDM	Experimental	TLR9 Expression	VitD decreases TLR9 expression in DVI
Lai et al., (2018)	Tao-Yuan, Taiwan	DC	Experimental	TLR9 expression and activation	DENV increases TLR9 expression and activation
Lai et al., (2021)	Tao-Yuan, Taiwan	Mo-DC	Experimental	TLR9 expression and activation with mechanism of action	DENV increases TLR9 expression and activation via cytidine/uridine monophosphate kinase 2 (CMPK2)
Parthasarathy et al., (2018)	Chennai, India	Corneal tissue	Analytical observational	TLR9 Expression	TLR9 expression was found in the

Author (year)	Geographical	Targets	Study design	Parameters	Outcome
					corneas of DVI patients
Balakrishna Pillai et al., (2020)	Puducherry, India	PBMC	Analytical Observational	TLR9 Expression	TLR9 expression is increased in DVI patients and is associated with clinical manifestations
Singh et al., (2021)	Uttar Pradesh, India	Blood	Analytical observational	TLR9 Polymorphism	There exists an association between TLR9 polymorphisms and usceptibility to developing DVI.
Torres et al., (2013)	Medellı´n, Colombia	Monocytes mDCs, and pDCs	Experimental	TLR9 Expression	The expression of TLR9 was shown to be elevated in mDC of patients with DF and DHF, as well as in pDC of DF patients but there was no observed increase in TLR9 expression in monocytes.

The study's findings indicated a notable rise in the expression of the TLR9 gene and the activation of TLR9 in DVI. The activation of TLR9 originates via the recognition of mitochondrial DNA (mtDNA).

2. Discussion

The expression of TLR9 is elevated in individuals with DVI. The release of mtDNA triggers the activation of TLR9.

TLR9 Expression in DVI

The study conducted Parthasarathy et al. (2018) demonstrated a notable elevation in the expression of TLR9 in the corneas of patients diagnosed with DVI. Nevertheless, the expression of TLR9 in patients with DVI exhibited variability. The range of differences is contingent on cell type and degree of the disease.

TLR9 expression in mDCs increases following DENV infection. TLR9 expression is enhanced in dengue fever patients' mDC, as well as TLR9 expression in plasmacytoid dendritic cells (pDC). TLR9 expression on pDC was not increased in dengue hemorrhagic fever (DHF) patients. (Torres et al., 2013).

Due to DENV infection, the production of mitochondrial reactive oxygen species (mtROS) and inflammasome modulates TLR9 expression. Infection with DENV causes the release of mtDNA in response to the formation of mtROS. The evidence that antioxidants inhibit mtDNA release supports the theory that reactive oxygen species induce mtDNA release. DENV is known to activate

the NLRP3 inflammasome, resulting in caspase-1 activation. The existing inflammasome induces the release of mtDNA. It is confirmed by the evidence that caspase-1 inhibitors reduce mtDNA release. Increased TLR9 expression is triggered by both oxidized and unoxidized mtDNA (Lai et al., 2018).

TLR9 expression in DVI was reduced by the presence of vitamin D (VitD). Vitamin D binds to VitD receptors. VitD complexes with the VitD receptor and then recruits the retinoid X receptor. This complex affects the expression of genes with the VitD response element, resulting in reduced ROS synthesis (Castillo et al., 2021). VitD inhibited miR-130-3p expression in DENV-infected MDMs. Reduced miR-130-3p expression inhibits TLR9 expression (Castillo & Urcuqui-Inchima, 2023).

Mechanism of TLR9 Activation in DVI

DENV is an RNA virus, whereas TLR9 is an endogenous TLR that recognizes DNA. Activation of TLR9 in DVI is not due to its recognition of DENV RNA but to its recognition of mtDNA. (Lai et al., 2018; Lai et al., 2021).

A disparity exists in the cellular response between human cell lines and primary cells during infection with DENV. Primary cells such as DC are not destroyed by DENV infection. DC becomes active and migrates towards the lymphoid organs following DENV infection. DCs that have been activated produce IFN I. The interaction between IFN I and the IFN receptor activates the Jak/Stat pathway, triggering the expression of interferon-stimulating genes such as the CMPK2 gene. The cytoplasmic CMPK2 protein is then transported to the mitochondria. An 8-hydroxy-20-deoxyguanosine is produced when CMPK2 induces oxidative stress in mitochondria, characterized by the formation of oxidative stress. mtROS initiates mtDNA release (Lai et al., 2021).

DENV infection stimulates protein kinase A (PKA). The relationship between PKA and Transcription Factor A Mitochondria (TFAM) is weakened by PKA activation. This loosening permits the discharge of mtDNA (Lai et al., 2018).

The conducted scoping review features both limitations and implications. One noticeable constraint of the analysis is its need for a comprehensive examination of the therapeutic potential of TLR9 as a target for DVI. The implication of the involvement of TLR9 in DVI suggests that augmenting TLR9 expression has promise for the eradication of DENV.

CONCLUSION

The expression TLR9 exhibits an increase in moderate DVI, whereas it shows a reduction in severe DVI. The activation of TLR9 within the DVI occurs after the recognition of mitochondrial DNA (mtDNA) by TLR9. The release of mtDNA into the cytosol is caused by the activation of the protein kinase A (PKA)-TFAM pathway.Considering the constraints and consequences of the scoping review undertaken, it is imperative to initiate further investigation into the viability of utilizing TLR9 activation as a focal point for therapeutic interventions in the setting of DVI.

ACKNOWLEDGEMENTS

The authors acknowledge respect to the library staff at the Faculty of Medicine, Universitas Muhammadiyah Surakarta, for their assistance in conducting literature searches and preparing relevant materials.

REFERENCES

Arksey, H., & O'Malley, L. (2005). Scoping studies: Towards a methodological framework. *International Journal of Social Research Methodology: Theory and Practice*, *8*(1), 19–32. https://doi.org/10.1080/1364557032000119616

Balakrishna Pillai, A., Cherupanakkal, C., Immanuel, J., Saravanan, E., Eswar Kumar, V., A, A., Kadhiravan, T., & Rajendiran, S. (2020). Expression Pattern of Selected Toll-like Receptors (TLR's) in the PBMC's of Severe and Non-severe Dengue Cases. *Immunological Investigations*, *49*(4), 443–452. https://doi.org/10.1080/08820139.2019.1653908

Balka, K. R., & De Nardo, D. (2019). Understanding early TLR signaling through the Myddosome. *Journal of Leukocyte Biology*, *105*(2), 339–351. https://doi.org/10.1002/JLB.MR0318-096R

Castillo, J. A., Giraldo, D. M., Hernandez, J. C., Smit, J. M., Rodenhuis-Zybert, I. A., & Urcuqui-Inchima, S. (2021). Regulation of innate immune responses in macrophages differentiated in the presence of vitamin D and infected with dengue virus 2. *PLoS Neglected Tropical Diseases*, *15*(10), e0009873. https://doi.org/10.1371/journal.pntd.0009873

Castillo, J. A., & Urcuqui-Inchima, S. (2023). Vitamin D modulates inflammatory response of DENV-2-infected macrophages by inhibiting the expression of inflammatory-liked miRNAs. *Pathogens and Global Health*, *117*(2), 167–180. https://doi.org/10.1080/20477724.2022.2101840

Chao, C. H., Wu, W. C., Lai, Y. C., Tsai, P. J., Perng, G. C., Lin, Y. S., & Yeh, T. M. (2019). Dengue virus nonstructural protein 1 activates platelets via Toll-like receptor 4, leading to thrombocytopenia and hemorrhage. *PLoS Pathogens*, *15*(4), e1007625. https://doi.org/10.1371/journal.ppat.1007625

Chen, J., Ng, M. M. L., & Chu, J. J. H. (2015). Activation of TLR2 and TLR6 by Dengue NS1 Protein and Its Implications in the Immunopathogenesis of Dengue Virus Infection. *PLoS Pathogens*, *11*(7), e1005053. https://doi.org/10.1371/journal.ppat.1005053

Cipitelli, M. da C., Amâncio Paiva, I., Badolato-Corrêa, J., & de-Oliveira-Pinto, L. M. (2019). Influence of chemokines on the endothelial permeability and cellular transmigration during dengue. *Immunology Letters*, *212*, 88–97. https://doi.org/10.1016/j.imlet.2019.06.001

El-zayat, S. R., Sibaii, H., & Mannaa, F. A. (2019). Toll-like receptors activation, signaling, and targeting: an overview. *Bulletin of the National Research Centre*, *43*, 187. https://doi.org/https://doi.org/10.1186/s42269-019-0227-2

Fernandes-Santos, C., & de Azeredo, E. L. (2022). Innate Immune Response to Dengue Virus: Toll-like Receptors and Antiviral Response. *Viruses*, *14*, 992. https://doi.org/10.3390/v14050992

Gay, N. J. (2019). Role of self-organising myddosome oligomers in inflammatory signalling by Toll-like receptors. *BMC Biology*, *17*, 15. https://doi.org/10.1186/s12915-019-0637-5

Guzman, M. G., & Harris, E. (2015). Dengue. *The Lancet*, *385*(9966), 453–465. https://doi.org/10.1016/S0140-6736(14)60572-9

Kayesh, M. E. H., Kohara, M., & Tsukiyama-Kohara, K. (2021). Recent Insights Into the Molecular Mechanism of Toll-Like Receptor Response to Dengue Virus Infection. *Frontiers in Microbiology*, *12*(74233), 1–13. https://doi.org/10.3389/fmicb.2021.744233

Kim, B., Lee, Y., Kim, E., Kwak, A., Ryoo, S., Bae, S. H., Azam, T., Kim, S., & Dinarello, C. A. (2013). The interleukin-1α precursor is biologically active and is likely a key alarmin in the IL-1 family of cytokines. *Frontiers in Immunology*, *4*(NOV), 1–9. https://doi.org/10.3389/fimmu.2013.00391

Kuczera, D., Assolini, J. P., Tomiotto-Pellissier, F., Pavanelli, W. R., & Silveira, G. F. (2018). Highlights for Dengue Immunopathogenesis: Antibody-Dependent Enhancement, Cytokine Storm, and beyond. *Journal of Interferon and Cytokine Research*, *38*(2), 69–80. https://doi.org/10.1089/jir.2017.0037

Lai, J.-H., Wang, M.-Y., Huang, C.-Y., Wu, C.-H., Hung, L.-F., Yang, C.-Y., Ke, P.-Y., Luo, S.-F., Liu, S.-J., & Ho, L.-J. (2018). Infection with the dengue RNA virus activates TLR9 signaling in human dendritic cells. *EMBO Reports*, *19*(8), e46182. https://doi.org/10.15252/embr.201846182

Lai, J.-H., Wu, D.-W., Wu, C.-H., Chen, A., Chang, Z.-F., Ho, L.-J., Hung, L.-F., Huang, C.-Y., & Ka, S.-M. (2021). Mitochondrial CMPK2 mediates immunomodulatory and antiviral activities through IFN-dependent and IFN-independent pathways. *Cell.Com*. https://doi.org/10.1016/j.isci.2021.102498

Malavige, G. N., & Ogg, G. S. (2017). Pathogenesis of vascular leak in dengue virus infection. *Immunology*, *151*, 261–269. https://doi.org/10.1111/imm.12748

Modhiran, N., Watterson, D., Blumenthal, A., Baxter, A. G., Young, P. R., & Stacey, K. J. (2017). Dengue virus NS1 protein activates immune cells via TLR4 but not TLR2 or TLR6. *Immunology and Cell Biology*, *95*(5), 491–495. https://doi.org/10.1038/icb.2017.5

Parthasarathy, D., Madhuravasal, J. K., Jayavel, P., Kulandai, L. T., Narahari Rao, M. H., & Jambulingam, M. (2018). Expression analysis of toll-like receptors of Dengue-infected cornea by real-time polymerase chain reaction. *Inflammation Research : Official Journal of the European Histamine Research Society ... [et Al.]*, *67*(7), 555–558. https://doi.org/10.1007/s00011-018-1148-5

Pawson, R. (2002). Evidence-based Policy : In Search of a Method. *Evaluation*, *8*(2), 157–181.

Singh, A., Prakash, S., Garg, R., Jain, P., Kumar, R., Cureus, A. J.-, & 2021, undefined. (2021). Study of single nucleotide polymorphisms in endosomal toll-like receptors-3, 7, and 9 genes in patients with dengue: A case-control study. *Cureus.Com*. https://doi.org/10.7759/cureus.14883

Sprokholt, J., Helgers, L. C., & Geijtenbeek, T. B. H. (2018). Innate immune receptors drive dengue virus immune activation and disease. *Future Virology*, *13*(4), 287–305. https://doi.org/10.2217/fvl-2017-0146

Torres, S., Hernández, J. C., Giraldo, D., Arboleda, M., Rojas, M., Smit, J. M., & Urcuqui-Inchima, S. (2013). Differential Expression of Toll-like Receptors in Dendritic Cells of Patients with Dengue during Early and Late Acute Phases of the Disease. *PLoS Neglected Tropical Diseases*, *7*(2), e2060. https://doi.org/10.1371/journal.pntd.0002060

Ullah, M. O., Sweet, M. J., Mansell, A., Kellie, S., & Kobe, B. (2016). TRIF-dependent TLR signaling, its functions in host defense and inflammation, and its potential as a therapeutic target. *Journal of Leukocyte Biology*, *100*(1), 27–45. https://doi.org/10.1189/jlb.2ri1115-531r

Wang, K., Zou, C., Wang, X., Huang, C., Feng, T., Pan, W., Wu, Q., Wang, P., & Dai, J. (2018). Interferon-stimulated TRIM69 interrupts dengue virus replication by ubiquitinating viral

nonstructural protein 3. *PLoS Pathogens*, *14*(8), e1007287. https://doi.org/10.1371/journal.ppat.1007287