AL-KAUNIYAH: Jurnal Biologi, 19(2), 2026, 253-261
Website: http://journal.uinjkt.ac.id/index.php/kauniyah
P-ISSN: 1978-3736, E-ISSN: 2502-6720

IMMUNOGLOBULIN M RESPONSE POST DBL2B-PFEMP1 RECOMBINANT PROTEIN INJECTION IN WISTAR RATS: STUDY FOR MALARIA VACCINE DEVELOPMENT

RESPONS IMMUNOGLOBULIN M PASCAINJEKSI PROTEIN REKOMBINAN DBL2B-PFEMP1 PADA TIKUS WISTAR: STUDI PENGEMBANGAN VAKSIN MALARIA

Erma Sulistyaningsih[1*], Achmad Faza Abdillah[2], Rosita Dewi[3], Sheilla Rachmania[3]
[1]*Department of Parasitology, Faculty of Medicine, Universitas Jember, East Java 68121*
[2]*Faculty of Medicine, Universitas Jember, East Java 68121*
[3]*Department of Histology, Faculty of Medicine, Universitas Jember, East Java 68121*
**Corresponding author:* sulistyaningsih.fk@unej.ac.id

Submitted: 11 January 2025; Revised: 28 September 2025; Accepted: 9 April 2026

Abstract

Vaccination is vital in malaria prevention strategies. The DBL2β-PfEMP1 recombinant protein is a malaria vaccine candidate due to its essential role in severe pathogenesis. This study analyzed the IgM levels after DBL2β-PfEMP1 recombinant protein injection. This study used nineteen male Wistar rats, aged 2–3 months, and was divided into a control group, which was injected with NaCl 0.9%, and four treatment groups, which were injected with DBL2β-PfEMP1 protein in doses of 150 µg without adjuvant, and 150, 300, and 450 µg with adjuvant. Rats were injected subcutaneously twice at a 6-week interval. Blood samples were collected two weeks after injection. IgM was measured using the ELISA. There was an increasing trend in IgM levels after the DBL2β-PfEMP1 protein injection with a dose-dependent effect in the primary injection. The One-way ANOVA test showed a significant difference between groups (p = 0.037), and the post-hoc Bonferroni test showed a significant difference only between a control and treatment group of 450 µg + adjuvant (p= 0.046). A trend of increasing IgM in secondary injection was only observed in the 150 µg + adjuvant group, which is suggested as an optimum dose. Further analysis of other immune indicators is needed to draw a comprehensive immune response in developing a malaria vaccine candidate.

Keywords: DBL; IgM; Malaria; PfEMP1; Vaccine

Abstrak

Vaksinasi berperan penting dalam strategi pengendalian malaria, namun vaksin malaria yang ada saat ini masih belum optimal. Protein rekombinan DBL2β-PfEMP1 merupakan kandidat vaksin karena peran pentingnya dalam patogenesis malaria berat. Tujuan penelitian ini adalah menganalisis kadar IgM pascainjeksi protein rekombinan DBL2β-PfEMP1. Penelitian ini menggunakan sembilan belas tikus Wistar jantan, berusia 2–3 bulan, yang secara random dibagi ke dalam kelompok kontrol, yang diinjeksi NaCl 0,9% dan empat kelompok perlakuan yang diinjeksi protein DBL2β-PfEMP1 dosis 150 µg, dan 150, 300, dan 450 µg plus adjuvant. Hewan coba diinjeksi secara subkutan sebanyak dua kali dengan interval 6 minggu. Sampel darah diambil setiap dua minggu setelah injeksi. Kadar IgM diukur menggunakan metode ELISA dan data dianalisis menggunakan uji One-Way ANOVA. Hasil penelitian menunjukkan terdapat tren peningkatan kadar IgM seiring dengan peningkatan dosis pada injeksi primer. Analisis statistik menunjukkan perbedaan antar kelompok (p= 0,037). Analisis selanjutnya menggunakan post hoc Bonferroni test menunjukkan perbedaan signifikan hanya antara kelompok kontrol dan perlakuan 450 µg plus adjuvant (p= 0,046.) Terdapat tren peningkatan kadar IgM pada injeksi sekunder dibandingkan dengan primer hanya pada kelompok dosis 150 µg plus adjuvant, yang mengindikasikan dosis optimum vaksin. Penelitian selanjutnya menggunakan indikator respons imun perlu dilakukan untuk mendapatkan gambaran komprehensif respons imun guna pengembangan vaksin malaria.

***Kata Kunci**: DBL; IgM; Malaria; PfEMP1; Vaksin*

Permalink/DOI: http://dx.doi.org/10.15408/kauniyah.v19i2.44265

INTRODUCTION

Malaria is one of the leading infectious diseases with high morbidity and mortality. The World Health Organization (WHO) estimated 249 million cases of malaria spread across 85 countries, with 608,000 deaths worldwide in 2022, and Indonesia is at the second rank of the highest number of cases and deaths in Southeast Asia, trailing India. *Plasmodium falciparum* is the main cause of malaria in Indonesia, contributing to 62% of all malaria cases (World Health Organization (WHO), 2023). It is responsible for severe malaria symptoms leading to death due to cytoadherence and rosetting mechanisms (Lee et al., 2019; Jensen et al., 2020).

One essential strategy to prevent and control malaria is vaccination (World Health Organization (WHO), 2024). Therefore, vaccine development is a vital pillar in achieving the target of the Global Technical Strategy for malaria 2030 (World Health Organization (WHO), 2021). Currently, two malaria vaccines are approved by WHO, i.e., the RTS, S/AS01 (Recombinant T-cell Epitopes and Surface Antigen) and R21 vaccines. Both vaccines use a circumsporozoite protein (CSP) as an immunogenic protein and work at the preerythrocytic phase (Hammershaimb & Berry, 2023). The RTS, S/AS01 vaccine is the first and foremost malaria vaccine with an efficacy of 56%, which is under the WHO minimum standard of 75% vaccine efficacy (Kurtovic et al., 2021; El-Moamly & El-Sweify, 2023). The second approved malaria vaccine is R21, with an efficacy of 77% (Datoo et al., 2021). However, both vaccines are targeted at the preerythrocytic phase and are not optimal in preventing the release of merozoites during malaria infection. Therefore, developing a malaria vaccine targeted at a different phase of parasites, such as the erythrocytic phase, is needed. One studied erythrocytic vaccine candidate is *Plasmodium falciparum* Erythrocyte Membrane Protein 1 (PfEMP1). This protein is secreted during the erythrocytic cycle and has a crucial role in severe malaria pathogenesis by its capacity to bind receptors on endothelial cells of capillaries (Sulistyaningsih et al., 2020).

Plasmodium falciparum Erythrocyte Membrane Protein 1 (PfEMP1) can be found on the surface of infected erythrocytes. It is a complex protein consisting of extracellular and intracellular parts (Wiser, 2023). The extracellular part of PfEMP1 is composed of two main domains, i.e., Duffy Binding-like (DBL) region and Cysteine-rich interdomain region (CIDR) (Tessema et al., 2018). The DBL domain is consistently found in the PfEMP1 structure and classified into seven classes, namely α, ß, γ, δ, ξ, ε, and x. The DBL2ß domain mediates binding to the intercellular adhesion molecule-1 (ICAM-1) receptor, leading to severe malaria pathogenesis (Bachmann et al., 2019; Jensen et al., 2020; Gill et al., 2023). Thus, the DBL2ß-PfEMP1 is a potential malaria vaccine candidate.

Previous studies have shown that the DBL2β-PfEMP1 recombinant protein could induce IgG antibodies and CD4+ lymphocyte production (Rachmania et al., 2020). It is known that humoral and cellular immune responses are involved in malaria infection (López et al., 2017; Sebina & Pepper, 2018). Humoral immunity is essential in controlling malaria infection, but malaria infection causes changes in B cell activity, resulting in a non-optimal response (Rogers et al., 2021). However, a study showed increased immunoglobulin M (IgM) levels after injection of MSP1 recombinant protein (Blank et al., 2020).

Immunoglobulin M is the first response in humoral immunity (Ji et al., 2023). In malaria infection, IgM works by opsonizing sporozoites to destroy them and preventing the merozoites invasion into erythrocytes by using a complement system to control malaria infections (Pleass et al., 2016; Boyle et al., 2019). Increased IgM levels in the blood will protect the body against infection and control the degree of parasitemia (Boonyaratanakornkit & Taylor, 2019). A study in the RTS, S vaccine also showed that an increase in IgM is associated with control of the parasitemia degree after RTS, S/AS01 injection (Kurtovic et al., 2021). This study analyzed the IgM response after injection of DBL2β-PfEMP1 recombinant protein in Wistar rats.

MATERIALS AND METHODS

Samples and Ethical Clearance

The samples of this study were male Wistar rats (*Rattus Norvegicus*), aged 2–3 months, with a weight between 150–250 g. This study has received permission from the Research Ethics Committee of the Faculty of Medicine, Universitas Jember, No. 5080/UN25.1.10.2/KE/2024.

Production and Purification of DBL2β-PfEMP1 Recombinant Protein

The DBL2β-PfEMP1 recombinant protein was expressed in *Escherichia coli* BL21 (DE3) (Hasanah et al., 2020). The starter bacteria were grown in 5 mL of Luria Bertani (LB) Broth (Merck, catalog number 110285), supplemented with kanamycin (Himedia, catalog number TC138), and incubated in an orbital-shaker incubator at 37 °C with 160 rpm for 16 hours. The starter was transferred into 250 mL of LB Broth and incubated at 190 rpm for 4 hours until it reached an optical density (OD) of 0.8. The bacteria were induced with 0.5 mM IPTG (Promega, catalog number V3951) and incubated for 6 hours. The cultures were harvested by centrifugation at 6,000 rpm for 15 minutes. The recombinant protein was extracted by sonication, with an extraction buffer containing

NaCl 500 mM (Supelco, catalog number 15513), NaH_2PO_4 50 mM (Supelco, catalog number 106346), and imidazole 5 mM (Sigma, catalog number 15513) in pH 8.0 (Rachmania et al., 2020). The purification was performed using affinity chromatography with a Ni-NTA expressionist kit (Genscript, catalog number L00223). GenScript High-Affinity Ni-Charged Resin contains Ni^{2+} and is compatible and used in protein purification by four coordination sites for high-affinity purification of polyhistidine-tagged recombinant proteins. The recombinant protein was eluted using a serial elution buffer containing 100 mM and 150 mM imidazole. The protein concentration was measured using the Bradford protein assay and then visualized using SDS-PAGE.

Injection of DBL2β-PfEMP1 Recombinant Protein in Wistar Rats

The rats were acclimatized for 14 days with standard nutrition and *ad libitum*. Nineteen male Wistar rats, aged 2–3 months, were divided into five groups, i.e., the control group, which consisted of 3 Wistar rats, and four treatment groups, each consisting of 4 Wistar rats. The control group was injected with normal saline. The treatment groups were injected with DBL2β-PfEMP1 recombinant protein in a dose of 150 μg, 150 μg plus adjuvant, 300 μg plus adjuvant, and 450 μg plus adjuvant. Injections were performed subcutaneously, twice at 6-week intervals. A Complete Freund's adjuvant was used in the first injection, and an incomplete Freund's adjuvant in the secondary injection using a 1:1 ratio. Blood samples were collected from each group from retro-orbital two weeks after each injection of recombinant protein.

IgM Concentration Measurement with ELISA

The IgM concentration was measured from rat sera using competitive enzyme-linked immunosorbent assays (ELISA) methods. As much as 50 μL rat sera was added to the rat IgM antibody coated well, then 50 μL of biotinylated antigen was added. The plates were incubated for 60 minutes at 37 °C and washed 5 times using the wash buffer. An avidin-HRP was added and incubated for 60 minutes at 37 °C. As much as 50 μL substrate solution A and substrate solution B were added to each well, then incubated for 10 minutes at 37 C in the dark. Finally, 50 μL stop solution was added, and the absorbance was measured using a microplate reader at λ of 450 nm.

Statistical Analysis

Data analysis was carried out using the Statistical Package for the Social Sciences (SPSS) version 26.0 software. The Saphiro-Wilk test was used to test the data's normality, and the Levene test was used to test the data's homogeneity. The statistical analysis was performed using the One-Way ANOVA test to compare between groups and followed by post-hoc Bonferroni test with a 95% confidence interval.

RESULTS

It is yielded from the elution buffer 2 with 150 mM imidazole concentration (Figure 1). The measurement of DBL2β2-PfEMP1 protein concentration using the Bradford protein assay yielded 2 µg/µL, from a standard curve equation y = 0.0078x - 0.0703 with an R^2 value of 0.9962 and the average absorbance of 0.166 after three replications.

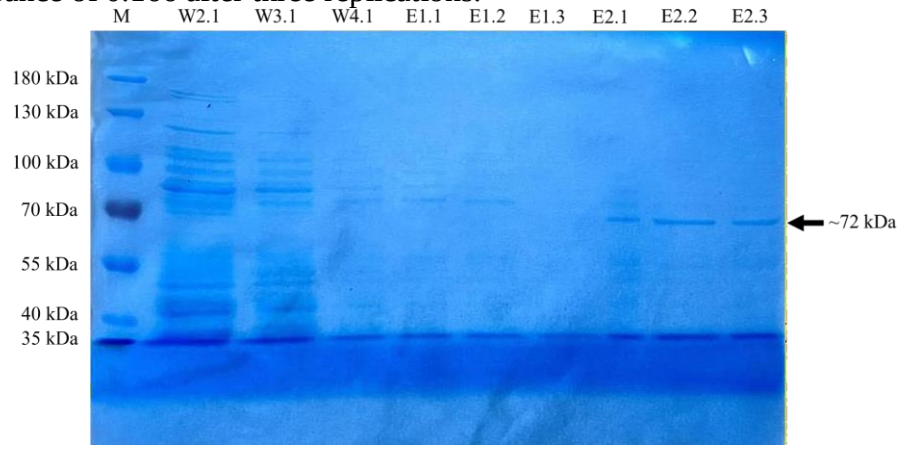

Figure 1. The visualization of DBL2β-PfEMP1 recombinant protein using SDS-PAGE. The purified DBL2β-PfEMP1 recombinant protein is ~72 kDa. M= protein marker; W2.1= protein fraction 1 from washing buffer 2; W3.1= protein fraction 1 from washing buffer 3; W4.1= protein fraction 1 from washing buffer 4; E1.1= protein fraction 1 from elution buffer 1; E1.2= protein fraction 2 from elution buffer 1; E1.3= protein fraction 3 from elution buffer 1; E2.1= protein fraction 1 from elution buffer 2; E2.2= protein fraction 2 from elution buffer 2; E2.3= protein fraction 3 from elution buffer 2

The measurement of IgM levels is presented in Figure 2. There is a trend of increasing IgM levels after DBL2β-PfEMP1 recombinant protein injection with a dose-dependent effect, meaning the higher the protein dose causing the higher the IgM level in sera, especially in the primary injection. But, for the secondary injection, a 450 µg plus adjuvant group showed a decrease in IgM level compared to a lower dose. The statistical analysis using the One-Way ANOVA test showed a significant difference between groups in primary injection with a p-value of 0.037 (Table 1). Further analysis using the post-hoc Bonferroni test showed a significant difference only between the treatment group at a dose of 450 µg plus adjuvant and the control group, with a p-value of 0.046.

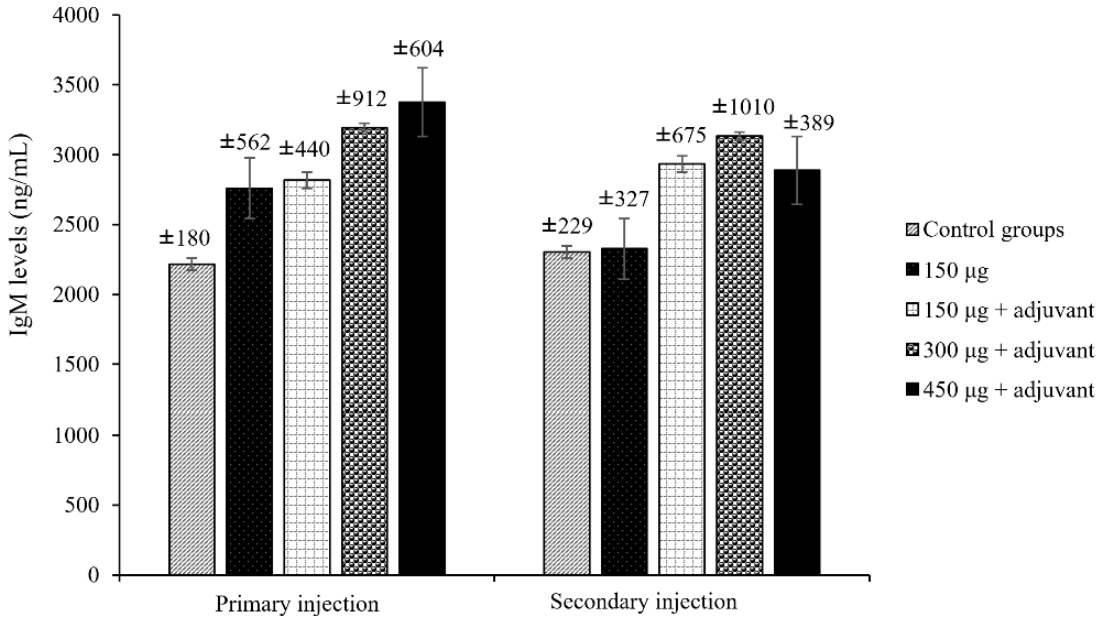

Figure 2. Histogram of IgM levels in the control and treatment groups after primary and secondary DBL2β-PfEMP1 recombinant protein injection. *= significant difference (P <0.05) based on post-hoc Bonferroni test

Table 1. The result of the first injection statistical analysis using One-Way ANOVA followed by the post hoc Bonferroni test

	Control	150 µg	150 µg + adjuvant	300 µg + adjuvant	450 µg + adjuvant	p-value
Control		1.000	1.000	0.198	0.046*	
150 µg	1.000		1.000	1.000	0.473	
150 µg + adjuvant	1.000	1.000		1.000	0.520	
300 µg + adjuvant	0.198	1.000	1.000		1.000	
450 µg + adjuvant	0.046*	0.473	0.520	1.000		0.037

Note: *=significant differences (P <0.05)

The comparison of IgM levels after the primary and secondary injections showed a different trend in each group (Figure 3). The increased trend was observed in the treatment group with a dose of 150 µg plus adjuvant and control groups, but other groups showed decreased IgM levels after secondary injection. The One-way ANOVA test showed no significant difference in all groups between the primary and secondary injections, with a p-value of 0.107.

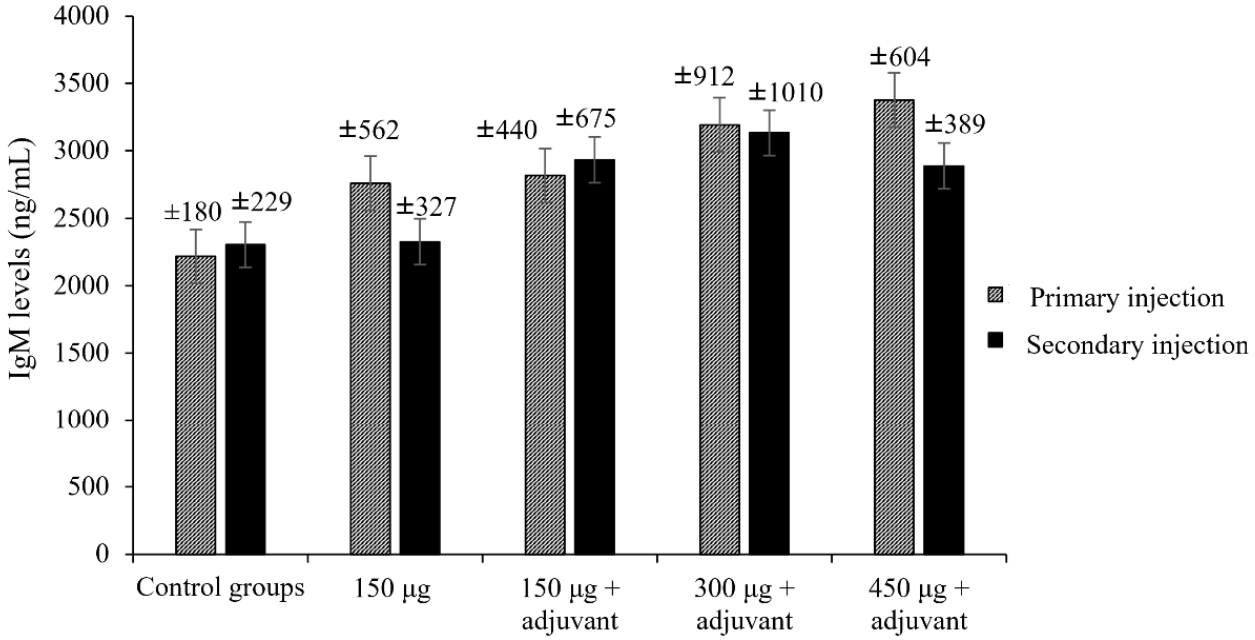

Figure 3. Histogram of IgM levels in each group after primary and secondary DBL2β-PfEMP1 recombinant protein injection

DISCUSSION

Malaria vaccine development is challenging due to the low efficacy of protection against malaria infection (Phillips et al., 2017). Natural immunity to malaria is commonly acquired slowly and needs repeated parasite exposure, and this immunity does not reduce parasitemia. The defense mechanisms need cooperation between the humoral immune response, which is played by antibodies and the cellular immune response, which involves T cells. Several antibodies such as IgE, IgM, and IgG are involved in malaria protection. The role of IgE antibody in malaria showed duality and complexity. Study showed that IgE serves to protect against the disease: other studies found a detrimental role during malaria disease (Blank & Mecheri, 2011). IgM is a vital and functional antibody that is initially and rapidly produced, activates complement, and reduces the risk of clinical malaria. It commonly acts as an early responder and is highly effective is blocking parasite invasion in the presence of complement (Boonyaratanakornkit & Taylor, 2019). While IgG acts as a long-live, secondary response, with cytophilic mechanisms, such as dependent cellular cytotoxicity (ADCC) and phagocytosis to control parasitemia (Stanisic et al., 2009). The vaccine candidate is usually added with adjuvants to enhance the immune response. While IgM is the first immunoglobulin produce by antibody-producing B-cells during an initial induction and is a stronger activator of complement, adjuvants accelerate the maturation, affinity, and quantity of the further IgG response by modulating

cytokine networks, optimizing antigen delivery, and stimulating Pattern Recognition Receptors (PRRs) on Antigen-Presenting cells (APCs) (Pedersen et al., 2020). Furthermore, adjuvants shorten the time for the body to produce antibody (Chang et al., 2017). Several factors contributing to the IgM response including a prior exposure, genetic factors, age, duration of exposure, antigen specificity, and parasitemia level. By adding adjuvants, the IgM response is also influenced by adjuvant type, antigen dose, and route of administration. The DBL2β-PfEMP1 recombinant protein is one of the malaria vaccine candidates currently being developed with the ability to induce IgG and CD4+ lymphocyte cells in Wistar rats (Rachmania et al., 2020). This study further analyzed the humoral immune response by measuring IgM levels using different doses of the DBL2β-PfEMP1 recombinant protein. Humoral immune response relies on antibody production B cells against extracellular pathogens, especially surface antigens of infected erythrocytes. When an antigen is recognized and internalized by the IgM B cell receptor, the B cell will differentiate and program to down regulate surface IgM and secrete either class-switched (IgG) or un-switched (IgM) antibody. B cell derived IgM is designed to provide rapid, short-lived, and protective immunity. Other B cells engage with T helper cells and reinforce the B cell and T follicular helper cells, promote class-switch recombination from IgM to IgG and stimulate co-migration of B and T cells to establish germinal center responses. This will promote B-cell survival and differentiation of memory B cells and long-lived plasma cells. The plasma cell constitutively secretes high-affinity IgG and memory B cell rapidly differentiate into high-affinity antibody following antigen exposure, which form the basis of long-lived protective immunity (Rogers et al., 2021).

The DBL2β-PfEMP1 recombinant protein was purified, visualized, and measured to ensure the accurate protein and precise dose injected in experimental animals. The purified DBL2β-PfEMP1 recombinant protein is ~72 kDa as in a previous study (Rachmania et al., 2020) with a 2 μg/μL concentration. The purified protein yielded from the second elution buffer with an imidazole concentration of 150 mM (Figure 1). The confirmed DBL2β-PfEMP1 recombinant protein was injected into experimental animals to determine its effect on IgM levels.

This study showed an increase in IgM levels in the treatment group compared to the control group, which had a dose-dependent effect after primary injection. The statistical analysis supported the results, which showed a significant difference between groups, especially between the control and 450 μg plus adjuvant group (Figure 2; Table 1). The results align with the study on reticulocyte-binding protein homologous protein-5 (RH5) recombinant protein injection, which showed an increase in antibody production (Minassian et al., 2021). The IgM levels in each group changed between primary and secondary injections. The increased IgM levels were observed in the control and treatment groups of 150 μg plus adjuvant, while other groups showed a decrease (Figure 3). However, the statistical analysis showed no significant ($p = 0.107$). The results align with the immune response paradigm that the IgM response is optimum at two weeks after primary exposure and decreases after the following exposure (Gong & Ruprecht, 2020).

Immunoglobulin M is the first humoral adaptive immune response produced when an antigen enters the body (Gong & Ruprecht, 2020). Determination of IgM is essential in vaccine development. In malaria vaccine injection, the resulting immune response involves an innate and adaptive immune response. The innate immune response is played by dendritic cells and macrophages that serve as antigen-presenting cells (APCs). Macrophages and dendritic cells will recognize incoming antigens through pattern recognition receptors (PRRs), presenting these antigens to CD8+ and CD4+ T cells through T cell receptors (TCRs) using major histocompatibility complex (MHC) molecules. The adaptive immune response involves the T cell activation that induces cytokines production such as interferon-γ (IFN-γ), interleukin-12 (IL-12), and tumor necrosis factor-α (TNF-α). Activated T cells can stimulate the activation of B cells through B cell receptors (BCRs), resulting in B cell maturation and antibody production, including IgM (Riley & Stewart, 2013; Nindela, 2015; Pollard & Bijker, 2021). The known conceptual theory for the humoral immune response to infection or vaccination is that the IgM peaks at two weeks after infection or immunization and wanes rapidly during the primary response, while IgG produced by isotype-switched memory B cells dominates in the secondary response (Gong & Ruprecht, 2020). However, Boonyaratanakornkit and Taylor (2019) showed

evidence of the role of IgM in both early and long-lived immunity to malaria, resulting in higher IgM levels after secondary injection or exposure. The high level of IgM in secondary exposure is suggested to be caused by the improved ability of IgM to fix complement to protein or parasite. It may be preserved in the secondary immune response to help protect against and control parasitemia, as a study in malaria infection showed IgM works by opsonizing the target protein and destroying it through complement-mediated lysis (Boyle et al., 2019). Furthermore, in the RTS, S'AS01 vaccine study, the increase in IgM levels was related to the ability to control the degree of parasitemia (Kurtovic et al., 2021). That finding supports our study results that there is an increase of IgM level in the 150 μg plus adjuvant group, which is expected to be the optimum dose of DBL2β-PfEMP1 protein as a malaria vaccine candidate.

CONCLUSION

The DBL2β-PfEMP1 recombinant protein injection increased IgM levels in Wistar rats with a dose-dependent effect, especially in the primary injection. Further studies on other immune response indicators should be conducted to elucidate a comprehensive immune response in order to establish a malaria vaccine.

ACKNOWLEDGMENTS

The authors thank the University of Jember for funding this study through an internal research grant for the Malaria Research Group in 2024.

REFERENCES

Bachmann, A., Bruske, E., Krumkamp, R., Turner, L., Wichers, J. S., Petter, M., ... Tannich, E. (2019). Controlled human malaria infection with *Plasmodium falciparum* demonstrates the impact of naturally acquired immunity on virulence gene expression. *PLOS Pathogens*, *15*(7), e1007906. doi: 10.1371/journal.ppat.1007906.

Blank, A., Fürle, K., Jäschke, A., Mikus, G., Lehmann, M., Hüsing, J., ... Bujard, H. (2020). Immunization with full-length *Plasmodium falciparum* merozoite surface protein 1 is safe and elicits functional cytophilic antibodies in a randomized first-in-human trial. *Npj Vaccines*, *5*(1). doi: 10.1038/s41541-020-0160-2.

Blank, U., & Mecheri, S. (2011) Duality and complexity of allergic type inflammatory mechanisms in determining the outcome of malaria disease. *Frontiers in Immunology*, *2*(78), 1-7. doi: 10.3389/fimmu.201100078.

Boonyaratanakornkit, J., & Taylor, J. J. (2019). Immunoglobulin M, more than just an early responder to malaria. *Immunology and Cell Biology*, *97*(9). doi: 10.1111/imcb.12292.

Boyle, M. J., Chan, J. A., Handayuni, I., Reiling, L., Feng, G., Hilton, A., … Beeson, J. G. (2019). IgM in human immunity to *Plasmodium falciparum* malaria. *Science Advances*, *5*(9), eaax4489. doi: 10.1126/sciadv.aax4489.

Chang, T. Z., Diambou, I., Kim, J. R., Wang, B., & Champio, J. A. (2017). Host-and pathogen-derived adjuvant coating on protein nanoparticle vaccines. *Bioengineering & Translational Medicine*, *2*(1), 120-130. doi: 10.1002/btm2.10052.

Datoo, M. S., Natama, M. H., Somé, A., Traoré, O., Rouamba, T., Bellamy, D., … Tinto, H. (2021). Efficacy of a low-dose candidate malaria vaccine, R21 in adjuvant Matrix-M, with seasonal administration to children in Burkina Faso: A randomised controlled trial. *The Lancet*, *397*(10287), 1809-1818. doi: 10.1016/S0140-6736(21)00943-0.

El-Moamly, A. A., & El-Sweify, M. A. (2023). Malaria vaccines: The 60-year journey of hope and final success—lessons learned and future prospects. *Tropical Medicine and Health*, *51*(1). doi: 10.1186/s41182-023-00516-w.

Gill, J., Singh, H., & Sharma, A. (2023). Profiles of global mutations in the human intercellular adhesion molecule-1 (ICAM-1) shed light on population-specific malaria susceptibility. *BMC Genomics*, *24*(1). doi: 10.1186/s12864-023-09846-9.

Gong, S., & Ruprecht, R. M. (2020). Immunoglobulin M: An ancient antiviral weapon – rediscovered. *Frontiers in Immunology*, *11*(1943). doi: 10.3389/fimmu.2020.01943.

Hammershaimb, E. A., & Berry, A. A. (2023). Pre-erythrocytic malaria vaccines: RTS,S, R21, and beyond. *Expert Review of Vaccines*, *23*(1), 49-52. doi: 10.1080/14760584.2023.2292204.

Hasanah, F. H., Sulistyaningsih, E., & Sawitri, W. D. (2020). The expression of the PfEMP1-DBL2β recombinant protein of *Plasmodium falciparum* Welch, 1897 isolated from Indonesia. *Jurnal Ilmu Dasar, 21*(1), 67. doi: 10.19184/jid.v21i1.10494.

Jensen, A. R., Adams, Y., & Hviid, L. (2020). Cerebral *Plasmodium falciparum* malaria: The role of PfEMP1 in its pathogenesis and immunity, and PfEMP1-based vaccines to prevent it. *Immunological Reviews*, *293*(1) 230-252. doi: 10.1111/imr.12807.

Ji, C., Shen, H., Su, C., Li, Y., Chen, S., Sharp, T. H., & Xiao, J. (2023). *Plasmodium falciparum* has evolved multiple mechanisms to hijack human immunoglobulin M. *Nature Communications, 14*(1). doi: 10.1038/s41467-023-38320-z.

Kurtovic, L., Atre, T., Feng, G., Wines, B. D., Chan, J. A., Boyle, M. J., … Beeson, J. G. (2021). Multifunctional antibodies are induced by the RTS,S malaria vaccine and associated with protection in a phase 1/2a trial. *Journal of Infectious Diseases*, *224*(7), 1128-1138. doi: 10.1093/infdis/jiaa144.

Lee, W. C., Russell, B., & Rénia, L. (2019). Sticking to a cause: The *Falciparum* malaria parasites cytoadherence paradigm. *Frontiers in Immunology*, *10*(1444). doi: 10.3389/fimmu.2019.01444.

López, C., Yepes-Pérez, Y., Hincapié-Escobar, N., Díaz-Arévalo, D., & Patarroyo, M. A. (2017). What is known about the immune response induced by *Plasmodium vivax* malaria vaccine candidates?. *Frontiers in Immunology*, *8*(126). doi: 10.3389/fimmu.2017.00126.

Minassian, A. M., Silk, S. E., Barrett, J. R., Nielsen, C. M., Miura, K., Diouf, A., … Draper, S. J. (2021). Reduced blood-stage malaria growth and immune correlates in humans following RH5 vaccination. *Med*, *2*(6), 701-719.e19. doi: 10.1016/j.medj.2021.03.014.

Nindela, R. (2015). Merozoite surface protein-1 (MSP-1) dan merozoite surface protein-2 (MSP-2) *Plasmodium falciparum* sebagai kandidat vaksin malaria. *Majalah Kedokteran Sriwijaya*, *47*(1). doi: 10.36706/mks.v47i1.2745.

Pedersen, G. B., Worzner, K., & Andersen, P. (2020). Vaccine adjuvants differentially affect kinetics of antibody and germinal center responses. *Frontiers in Immunology,* (11). doi: 10.3389/fimmu.2020.579761.

Phillips, M. A., Burrows, J. N., Manyando, C., van Huijsduijnen, R. H., Van Voorhis, W. C., & Wells, T. N. C. (2017). Malaria. *Nature Reviews Disease Primers*, *3*(1), 17050. doi: 10.1038/nrdp.2017.50.

Pleass, R. J., Moore, S. C., Stevenson, L., & Hviid, L. (2016). Immunoglobulin M: Restrainer of inflammation and mediator of immune evasion by *Plasmodium falciparum* malaria. *Trends in Parasitology*, *32*(2), 108-119. doi: 10.1016/j.pt.2015.09.007.

Pollard, A. J., & Bijker, E. M. (2021). A guide to vaccinology: From basic principles to new developments. *Nature Reviews Immunology*, *21*(2) 83-100. doi: 10.1038/s41577-020-00479-7.

Rachmania, S., Sulistyaningsih, E., & Ratna Dewi, A. A. I. (2020). Recombinant DBL2β-PfEMP1 of the Indonesian *Plasmodium falciparum* induces immune responses in Wistar rats. *Journal of Taibah University Medical Sciences, 16*(3), 422-430. doi: 10.1016/j.jtumed.2020.12.007.

Riley, E. M., & Stewart, V. A. (2013). Immune mechanisms in malaria: new insights in vaccine development. *Nature Medicine*, *19*(2), 168-178. doi: 10.1038/nm.3083.

Rogers, K. J., Vijay, R., & Butler, N. S. (2021). Anti-malarial humoral immunity: The long and short of it. *Microbes and Infection, 23*(4-5). doi: 10.1016/j.micinf.2021.104807.

Sebina, I., & Pepper, M. (2018). Humoral immune responses to infection: Common mechanisms and unique strategies to combat pathogen immune evasion tactics. *Current Opinion in Immunology*, *51*(46-54). doi: 10.1016/j.coi.2018.02.001.

Stanisic, D. I., Richards, J. S., McCallum, F. J., Michon, P., King, C. L., Schoepflin, S., … Beeson, J. G. (2009). Immunoglobulin G subclass-specific responses against *Plasmodium falciparum* merozoite antigens are associated with control of parasitemia and protection from symptomatic illness. *Infection and Immunity Journal*, *77*(3), 1165-74. doi: 10.1128/IAI.01129-08.

Sulistyaningsih, E. (2020). *Vaksin malaria: Perkembangan generasi vaksin, kandidat protein untuk vaksin, dan tantangannya*. Jember: UPT Penerbitan Universitas Jember.

Tessema, S. K., Utama, D., Chesnokov, O., Hodder, A. N., Lin, C. S., Abby Harrison, G. L., … Gla, H. (2018). Antibodies to intercellular adhesion molecule 1-binding *Plasmodium falciparum* erythrocyte membrane protein 1-dbl are biomarkers of protective immunity to malaria in a cohort of young children from Papua New Guinea. *American Society for Microbiology, 86*(8). doi: 10.1128/IAI.

World Health Organization (WHO). (2021). Full evidence report on the RTS,S/AS01 malaria vaccine. Malaria Policy Advisory Group Meeting, Geneva. Retrieved from https://www.nitag-resource.org/sites/default/files/2022-05/Full-evidence-report-on-the-rtss-as01-malaria-vaccine-2021.pdf.

World Health Organization (WHO). (2023). WHO guidelines for malaria. WHO Global Malaria Programme. Geneva. Retrieved from https://iris.who.int/bitstream/handle/10665/379635/B09146-eng.pdf?sequence=1.

World Health Organization (WHO). (2024). Malaria vaccine: WHO position paper. Weekly epidemiological record. Retrieved from https://iris.who.int/bitstream/handle/10665/376738/WER9919-eng-fre.pdf?sequence=2

Wiser, M. F. (2023). Knobs, adhesion, and severe *Falciparum* malaria. *Tropical Medicine and Infectious Disease*, *8*(7). https://doi.org/10.3390/tropicalmed8070353.