Medical Laboratory Technology Journal

Received 2022-19-12; Revised 2023-03-02; Accepted 2023-03-03

Available online at : http://ejurnal-analiskesehatan.web.id

Correlation of Parasitaemia and Anemia in Mice Infected with *Plasmodium berghei* ANKA

Inas Hasna Azizah[1], Stevani Florentia Bahi[1], *Heny Arwati[1,2], Mufasirin[1,3]

[1]Master Program on Immunology, Postgraduate School, Universitas Airlangga, Indonesia. [2]Department of Medical Parasitology, Faculty of Medicine, Universitas Airlangga, Indonesia. [3]Department of Veterinary Parasitology, Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia. *Email: heny-a@fk.unair.ac.id
DOI: 10.31964/mltj.v8i2.494

Abstract: Malaria is an infectious disease caused by protozoa of the genus *Plasmodium* and transmitted through the bite of a female *Anopheles* mosquito. *Plasmodium berghei* ANKA is a species of rodent malaria parasite that is commonly used to study malaria pathology and the immune system against infections. Parasitaemia in malaria is the figure of malaria pathology due to some numbers of parasite-infected erythrocytes present in the peripheral blood. Hemoglobin (HGB) and hematocrit (HCT) levels are the parameters of anemia and some hematological changes caused by malaria infection. This study aimed to determine the correlation between parasitemia and anemia in BABL/c mice infected with *Plasmodium berghei* ANKA. Two uninfected and infected mice groups were compared for parasitemia, HGB, and HCT levels. Analysis statistics showed a significant difference in HGB and HCT between uninfected and infected groups. Pearson correlation analysis showed no significant correlation between parasitemia and HGB and HCT levels in infected mice. Anemia in mice infected with *Plasmodium berghei* ANKA can occur when parasitemia is even low; the higher parasitemia worsens the hamatological condition. Parasitemia plays a role independently in the severity of anemia. *Plasmodium berghei* infection in mice is useful for studying malaria anemia.
Keywords: Anemia; hemoglobin; hematocrit; parasitemia; *Plasmodium berghei* ANKA.

INTRODUCTION

Malaria is an infectious disease caused by protozoa of the genus *Plasmodium* and transmitted through the bite of a female *Anopheles* mosquito (Arwati et al., 2018). Rodent malaria parasites have been used extensively for malaria research (Craig et al., 2012; Otto et al., 2014). *Plasmodium berghei* ANKA is a species of rodent malaria parasites commonly used to study malaria pathology and the immune system against infections. *Plasmodium berghei* is an analogue infection that occurs in humans and primates. *Plasmodium berghei* is similar to the surface proteins that play a role in red blood cell invasion as observed from several aspects such as structure, physiology, morphology and life cycle. *Plasmodium berghei* infection can cause cerebral complications in experimental animals, such as cerebral malaria in humans infected with *P. falciparum* (Craig et al., 2012; LUMC, 2011). The *Plasmodium berghei* infection is also a good model for malaria anemia, which can provide a useful resource for further human malaria infection (Lakkavaram et al., 2020), which can be studied using BALB/c mice. The BALB/c mouse strain is commonly used to study human diseases, including malaria (Raz, 2022) and malaria

Corresponding Author: Heny Arwati
Department of Medical Parasitology, Faculty of Medicine, Universitas Airlangga,
Jl. Prof. Dr. Moestopo No. 47, Surabaya 60131, Indonesia.
Email: heny-a@fk.unair.ac.id

anemia (Lamikanra et al., 2007). BALB/c mice are susceptible to species of rodent malaria parasites, including *Plasmodium berghei* (Langhorne et al., 2002).

Parasitaemia in malaria is the figure of malaria pathology due to some numbers of parasite-infected erythrocytes present in the peripheral blood, sometimes with or without symptoms. Hemoglobin and hematocrit levels are the parameters of anemia which is some of the hematological changes caused by malaria infection (Awoke & Arota, 2019). Further, malaria anemia in humans that also occurs in mice may include the following mechanism: (1) the clearance and/or destruction of infected erythrocytes, (2) the clearance of uninfected erythrocytes, (3) erythropoietic suppression and dyserythropoietic (Lamikanra et al., 2007).

Severe malaria anemia in humans and mice is different because malaria anemia in humans can occur when acute malaria develops in low parasitemia. In contrast, acute infection in mice develops when parasitemia is high (Lamikanra et al., 2007; Evans et al., 2006). Several studies on malaria anemia in humans and mice have been reported, especially related to the immunity and pathogenesis (Perkins et al., 2011), immune response and genetic factors of the hosts (Helegbe et al., 2018), erythropoiesis (Lakkavaram et al., 2020; Pathak and Ghosh, 2016), and some reviews on the mechanism of anemia in human and mice (Lamikanra et al., 2007; Haldar and Mohandas, 2009). The effect of malaria on hemoglobin concentration among malaria-infected children (Starck et al., 2021) and the correlation of parasitemia and peripheral monocyte-to-lymphocyte ratio in severe malaria (Antwi-Baffour et al., 2018) have also been reported. However, very few studies have examined the correlation between parasitemia and anemia. Therefore, this study aims to describe the correlation between parasitemia and anemia, especially in the hemoglobin (HGB) and hematocrit levels in mice infected with *Plasmodium berghei* ANKA.

MATERIALS AND METHODS

Experimental protocol

The proposal of this study has been reviewed by the Health Research Ethical Clearance Commission, Faculty of Dental Medicine Universitas Airlangga, as described on certificate number 828/HRECC.FODM/XI/2022.

Twenty male BALB/c mice aged 7-8 weeks and weight ± 25 grams after one-week acclimatization were divided into two groups. Group 1 was a negative control group; they were not infected with *Plasmodium berghei* ANKA. Group 2 was infected with 1x106 of *P. beghei* ANKA-infected blood from mice donors. Parasitaemia was microscopically observed daily on a Giemsa-stained tail with thin blood smears (Arwati et al., 2021). Briefly, the tip of the tail was slightly cut using surgical scissors, and then one drop of blood was dropped on an object of glass to make a thin smear; after the thin smear was dry then, it was fixed with methanol and stained with 10% Giemsa solution for 10 minutes. Parasitemia was observed under 1,000 magnification of light microscopy using oil immersion. Parasitemia was counted based on the number of infected erythrocytes within 1,000 erythrocytes, as shown in the following formula:

$$\text{Parasitemia} = \frac{\text{Number of infected eryhrocytes}}{\text{Total number of infected eryhrocytes counts}} \text{X } 100$$

On day four post-infection, blood was taken from the tip of the tail of each mouse, as explained above prior, for hemoglobin (HGB) and hematocrit (HCT) levels measurement. The HGB and HCT levels were measured using chromatography-

based digital hemoglobin check equipment specific for blood (Fora 6, Switzerland). A drop of blood was dropped on the reaction area of the test strip; then, the strip was inserted into the equipment. The equipment will measure the HGB and HCT. Within 5 seconds, the results were displayed on the equipment's screen.

Statistical data analysis

The normality of data was analyzed using the Shapiro-Wilk test. When the data is distributed normally, the difference between the two experimental groups was analyzed using the independent t-test. The correlation between parasitemia and the level of HGB and HCT in Groups 1 and 2 was analyzed using the Pearson test. When data is not normally distributed, Mann Whitney U was applied. The correlation between parasitemia and HGB and HCT was analyzed using the Spearman correlation test. Statistical analysis was performed using the program SPSS version 25.

RESULTS AND DISCUSSION

Parasitaemia

Two out of 10 mice died on day three post-infection due to high parasitemia. Data on the average parasitemia of 8 mice in Group 2 during the four-day course of infection is presented in Figure 1. No parasitemia in mice of Group 1 because there was no infection. Parasitemia in Group 2 normally increased for the first-day post-infection and reached 57.44% on the fourth-day post-infection.

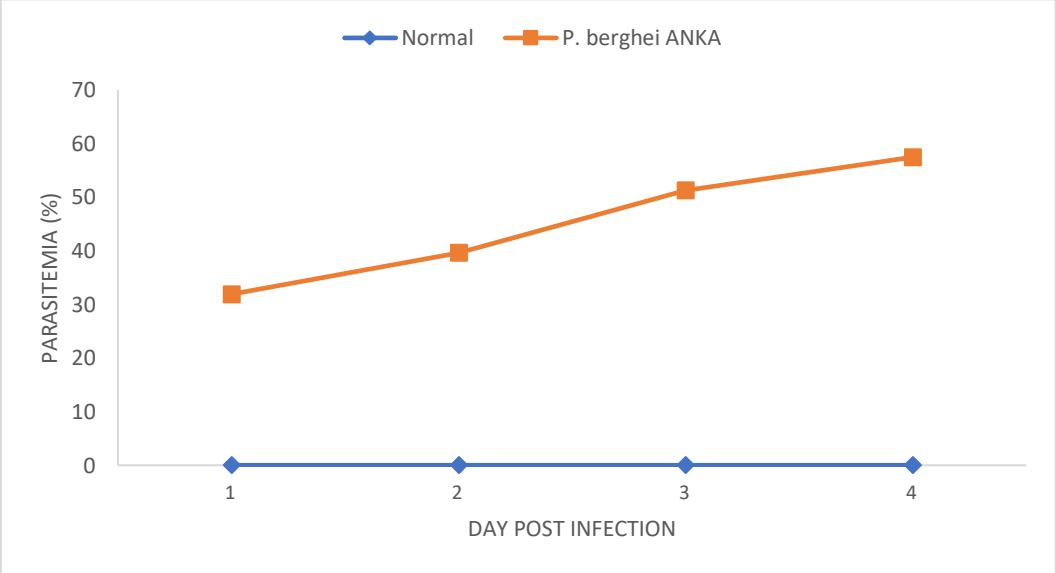

Figure 1. Parasitaemia of *Plasmodium berghei* ANKA infection in BALB/c mice.

Hemoglobin (HGB) and hematocrit (HCT) levels

The average HGB level of 8 mice in Groups 1 and 2 on day four post-infection is presented in Table 1. Hematological data of normal BALB/c mice showed that the normal HGB level was 12.6-20.5 g/dl, and the HCT level was 42.1-68.3 % (Charles River Research Models, 2019). The average level of HGB in uninfected mice of Group 1 was 12.67 g/dL, it was a normal level compared with that in infected mice of Group 2, which was 5.90 g/dL, and the average level of HCT in Group 1 showed a nearly normal level (37.75 %); however, mice in Group 2 showed a lower level of HCT (17.75%).

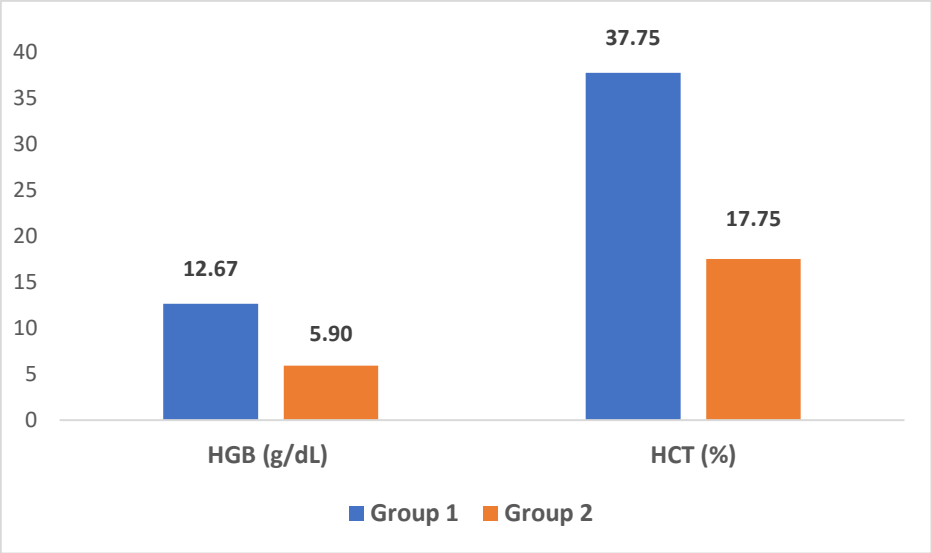

Figure 2. Hemoglobin (HGB) and hematocrit (HCT) levels of uninfected mice (Group 1) and *Plasmodium berghei* ANKA-infected mice (Group 2)

Statistical analysis

Analysis of normality data resulted in a normal distribution of data of either parasitemia, HGB and HCT. The independent T-test analysis of HGB level resulted in a significant difference (p=0.000) between infected (Group 2) and non-infected mice (Group 1) as well as HCT level (p=0.000). However, Pearson correlation analysis showed no significant correlation between parasitemia and HGB level (p=0.492) and parasitemia and HCT level (p=0.200) in Group 2.

The complexity of the relationship between parasitemia and clinical manifestations of malaria involves the nervous, respiratory, renal and/or hematopoietic systems (Cowell, 1969) (Trampuz et al., 2003). Malaria parasites consume erythrocytes' hemoglobin; therefore, they reduce the hemoglobin and hematocrit levels in malaria-infected mice due to the erythrocyte damage (Mohandas and An, 2012). The average level of HGB (5.90 g/dL) and HCT (17.75%) in mice infected with *Plasmodium berghei* ANKA (Group 2) were lower than HGB (12.67 g/dL) and HCT (37.75%) in uninfected mice (Group 1) (Charles River Research Models, 2019.). This data indicated the anemia status of mice infected with *Plasmodium berghei* ANKA in Group 2. These results are in line with control mice in a study of assessment of antimalarial medicinal plants showed that untreated *Plasmodium berghei*-infected mice had a mean HGB level was lower (19.7±1.68) than that of uninfected mice (73.8±2.00) (Ezeani et al., 2022); likewise, the HCT level in infected mice become lower than that of normal mice (Audomkasok et al., 2014; Somsak et al., 2015).

Microscopic observation of Giemsa-stained blood smears of mice infected with *Plasmodium berghei* showed that infected erythrocytes appeared in the peripheral blood in very low parasitemia at the beginning of the infection. As the parasitemia increased, the condition deteriorated. These conditions included decreasing erythrocytes, followed by an increasing number of reticulocytes. This condition may explain why parasitemia did not correlate significantly with the level of HGB and HCT. The *Plasmodium berghei* parasite infects erythrocytes and reticulocytes. If the reticulocytes were also infected, the condition worsened and

eventually resulted in death, as in the case of four infected mice during the four-day course of infection.

In this study, anemia in infected mice occurred when parasitemia was high. This is different from malaria anemia in humans. Severe malaria anemia in humans can occur when acute malaria is developed, even if parasitemia is low. Still, in the mouse malaria model, an acute infection developed where parasitemia can increase to more than 20% (Lamikanra et al., 2007). The course of infection and immunologic response of infection in the mouse malaria model always result in anemia (Lamb et al., 2006). The mechanism of malaria anemia in both humans and mice can be explained by the fact that during malaria infection, there is increased destruction of both infected and uninfected erythrocytes caused by membrane alterations of erythrocytes as well as erythropoiesis that cause anemia (Mohandas and An, 2012). Direct destruction of parasitized erythrocytes because of the rupture of erythrocytes infected with mature schizont stage and by macrophage-phagocytosis system in the spleen (Patel et al., 2004).

Anemia in humans as well as in mice has been explained by Pathak and Ghosh (2016) that anemia is not only due to the hemolysis of infected and uninfected erythrocytes but also due to the inability to replenish erythrocytes lost by hemolysis through inadequate erythroid response. The suppression of erythropoiesis is an additional factor contributing to anemia in humans and mice. Erythropoietic suppression and dyserythropoietic are underlying mechanisms of anemia in humans and mice (Lamikanra et a, 2007).

Severe anaemia's pathogenesis is multi-factorial, involving both host and parasite-mediated factors (Perkins et al., 2011). The growing evidence showed that anemia is not only caused by the clearance of erythrocytes but also by the inability of the host to produce an adequate erythroid response (Lamikanra et al., 2007). Immunological involvement of infected erythrocyte destruction is also an important factor in the pathogenesis of severe anemia (Arora et al., 2016). However, hemolysis of uninfected erythrocytes in human malaria is the more significant contributor to the rapid development of anemia and low hematocrit observed in patients with hyperparasitemia (Looareesuwan et al., 1987)(Dondorp et al., 2003).

The limitation of this study is that other anemia parameters were not analyzed due to the limited volume of blood used for other purposes.

CONCLUSIONS

In conclusion, anemia in mice infected with *Plasmodium berghei* ANKA can occur when parasitemia is even low; higher parasitemia worsens the hematological condition, such as the decrease in HGB and HCT. Parasitemia did not correlate significantly with HGB and HCT levels, probably due to increased circulating reticulocytes when parasitemia is high. The results of this study may increase the knowledge, especially on anemia related to parasitemia and the level of HGB and HCT. The pathogenesis of malaria anemia is multifactorial; therefore, research related to the factors involved in the pathogenesis of malaria anemia, such as immunological, nutritional, and even biochemistry aspects, must be conducted.

ACKNOWLEDGEMENT

This study is part of research supported by a grant from the Directorate General of Higher Education, Ministry of Education, Culture, Research and Technology of the Republic of Indonesia, decree number 1004/UN3/2022. We thank Mr Heri and Mr Choirul for taking care of the mice during the research.

CONFLICT OF INTEREST

All authors do not have any conflict of interest.

REFERENCES

Arora, G., Hart, G., & Desai, S. A and Long, E. (2022). Natural Killer Cell Antibody-Dependent Cellular Cytotoxicity (ADCC) Activity Against Plasmodium falciparum-Infected Red Blood Cells. *Methods Mol Biol*, *2470*, 641–657.

Arwati, H., Bahalwan, R., Hapsari, W., Wardhani, K., Aini, K., Apsari, P., & Wardhani, P. (2021). Suppressive effect of goat bile in Plasmodium berghei ANKA infection in mice. *Veterinary World*, *14*(8), 2016–2022.

Arwati, H., Yotopranoto, S., Rohmah, E. A., & Syafruddin, D. (2018). Submicroscopic malaria cases play role in local transmission in Trenggalek district, East Java Province, Indonesia. *Malaria Journal*, *17*(1). https://doi.org/10.1186/s12936-017-2147-7

Awoke, N., & Arota, A. (2019). Profiles of hematological parameters in Plasmodium falciparum and Plasmodium vivax malaria patients attending Tercha General Hospital, Dawuro Zone, South Ethiopia. *Infect Drug Resist.*, *12*, 521–527.

Brown, A., Webster, H., Teja-Isavadharm, P., & Keeratithakul, D. (1990). Macrophage activation in falciparum malaria as measured by neopterin and interferon-gamma. *Clin Exp Immunol.*, *1992*(82), 97–101.

Charles River Research Models. (2019). BALB/C Mouse Hematology. Retrieved from http://animalab.eu/sites/all/pliki/produkty-dopobrania/ balb_c_ Mouse_ clinical_ pathology_ data.pdf. Accessed on 20-8-2019.

Cowell, G. (1969). Relationship between Malarial Parasitaemia and Symptoms of the Disease. *Bull. Org. Mond. Sante | Bull. Wid Hith Org.*, *22*, 605–619.

Craig, A., EG, G., Janse, C., Kazura, J., Milner, D., Barnwell, J., … Jean, L. (2012). The Role of Animal Models for Research on Severe Malaria. *PLoS Pathog*, *8*(2), e1002401.

Dondorp, A., Omodeo-Sale, F., Chotivanich, K., & Taramelli, D. (2003). Oxidative stress and rheology in severe malaria. *Redox Rep. 2003*, *8*, 292–294.

Drakeley, C., Corran, P., Coleman, P., Tongren, J., SL, M., Carneiro, I., … Riley, E. (2005). Estimating medium- and long-term trends in malaria transmission by using serological markers of malaria expo sure. *Proc Natl Acad Sci U S A*, *102*, 5108–5113.

Evans, K., Hansen, D., van Rooijen, N., Buckingham, L., & Schofield, L. (2006). Severe malarial anemia of low parasite burden in rodent models results from accelerated clearance of uninfected erythrocytes. *Blood.*, *107*(3), 1192–1199.

Lakkavaram, A., Lundie, R., Do, H., Ward, A., & de Koning-Ward, T. (2020). Acute Plasmodium berghei Mouse Infection Elicits Perturbed Erythropoiesis With Features That Overlap With Anemia of Chronic Disease. *Front. Microbiol. 1*, *1*(702.).

Lamikanra, A., Brown, D., Potocnik, A., Casals-Pascua, C., Langhorne, J., & Roberts, D. (2007). Malarial anemia of mice and men. *Blood Volume*, *10*(1), 18–28.

Langhorne J, Quin SJ, Sanni LA. (2002). Mouse models of blood-stage malaria infections: immune responses and cytokines involved in protection and pathology. *Chem Immunol, 80*, 204-28. doi: 10.1159/000058845

Looareesuwan, S., Ho, M., Wattanagoon, Y., White, N., Warell, D., & Wyler, D. (1987). Dynamic alteration in splenic function during acute falciparum malaria. *N Engl J*

Med., *317*, 675-679.

LUMC. (2011). The Plasmodium berghei Research Model of Malaria. Retrieved May 20, 2011, from Centre, Leiden University Medical website: http://www.lumc.nl/1040/research/malaria/model01.html

Mohandas, N and An, X. (2012). Malaria and Human Red Blood Cells. *Med Microbiol Immunol.*, *201*(4), 593–598.

Otto, T., Böhme, U., Jackson, A., Hunt, M., Franke-Fayard, B., Hoeijmakers, W., … Janse, C. (2014). A comprehensive evaluation of rodent malaria parasite genomes and gene expression. *BMC Biology*, *12*(86).

Patel, S., Serghides, L., Smith, T., Febbraio, M., Silverstein, R., Kurtz, T., … Kain, K. (2004). CD36 mediates the phagocytosis of Plasmodium falciparum infected erythrocytes by rodent macrophages. *J. Infect. Dis. 1*, *89*, 204–213.

Pathak, VA and Ghosh, K. (2016). Erythropoiesis in Malaria Infections and Factors Modifying the Erythropoietic Response. *Anemia*, *2016*(9310905), 1–8.

Perkins, D., Were, T., Davenport, G., Kempaiah, P., Hittner, J., & and Ong'echa, J. (2011). Severe Malarial Anemia: Innate Immunity and Pathogenesis. *Int. J. Biol. Sci.*, *7*(9), 1427–1442.

Raz, A. (2022). Plasmodium berghei Infection in BALB/c Mice Model as an Animal Model for Malaria Disease Research. *Methods Mol Biol.*, *2410*, 589-595.

Trampuz, A., Jereb, M., Muzlovic, I., & Prabhu, R. (2003). Clinical review: Severe malaria. *Critical Care*, *7*(315–323).