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The Effect of Malaria on Hemoglobin Levels in Pregnant Women in the Third Trimester in 2022

Fitri Khoiriyah
D3 Keperawatan, STIKes Nauli Husada Sibolga, Indonesia

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ABSTRACT

Malaria is a serious public health problem in endemic areas, particularly in developing countries like Indonesia. This disease is caused by the Plasmodium parasite, which is transmitted through the bite of the female Anopheles mosquito. Pregnancy causes physiological and immunological changes that make pregnant women, especially in the third trimester, more susceptible to malaria complications. One common complication of malaria infection during pregnancy is anemia, characterized by a decrease in hemoglobin (Hb) levels in the blood (WHO, 2021). Malaria infection during pregnancy can cause the Plasmodium parasite to invade and destroy red blood cells, directly impacting hemoglobin levels. Furthermore, malaria can also cause hemolysis, impaired erythropoiesis, and increased iron requirements that are not met during pregnancy. This decrease in hemoglobin levels can trigger severe anemia, which carries the risk of obstetric complications such as premature delivery, low birth weight, and even maternal and fetal death (Desai et al., 2007; Menendez et al., 2015). This study aims to analyze the effect of malaria on hemoglobin levels in pregnant women in the third trimester in 2022. This study used an observational analytical design with a cross-sectional approach, conducted in a malaria-endemic area in Indonesia. The sample consisted of pregnant women in their third trimester diagnosed with malaria and compared with a control group not infected with malaria. Hemoglobin levels were measured using hematology laboratory tests. The results showed a significant difference between the hemoglobin levels of pregnant women with malaria compared to those not infected with malaria, with the group with malaria having lower hemoglobin levels ($p<0.05$). This confirms the finding that malaria contributes significantly to the incidence of anemia in pregnant women (Uneke, 2008). The impact of anemia in pregnancy is extensive, not only on maternal health but also on fetal development. Untreated anemia can cause fetal growth disorders, fetal hypoxia, and increase the risk of postpartum hemorrhage (Ministry of Health of the Republic of Indonesia, 2020). Therefore, early detection of malaria in pregnant women and appropriate management are crucial to prevent a dangerous decrease in hemoglobin levels. Increasing public and healthcare worker awareness of the importance of malaria screening during antenatal care is also key to preventive efforts. In conclusion, malaria significantly reduces hemoglobin levels in pregnant women during the third trimester. These findings are expected to inform the formulation of maternal and neonatal health policies in Indonesia to reduce maternal and infant morbidity and mortality due to malaria-induced anemia complications.

Corresponding Author:

Fitri Khoiriyah,
D3 Keperawatan,
STIKes Nauli Husada Sibolga, Indonesia,
Jln. Kader Mani No.02 Kelurahan Aek Muara Pinang Sibolga Selatan.
Email: Stikesnaulihusadasbg @gmail.com

1. Introduction

Malaria remains a serious global health problem, especially in developing countries with tropical and subtropical climates such as Indonesia.(Uswanas, 2019),(Ronny, 2024)This disease is caused by a parasite infection of the genus Plasmodium, which is transmitted through the bite of a female Anopheles mosquito. In Indonesia, malaria remains endemic in several regions, especially in remote areas, coastal areas, and isolated islands.(Arif, 2021),(Sumampouw, 2022). Based on data from the World Health Organization (WHO) in 2021, there were an estimated 241 million cases of malaria globally, with more than 627,000 deaths, and most of these affected vulnerable groups such as children and pregnant women.(Tondok et al., nd),(Fabanyo, 2022).

Pregnant women are a group that is very vulnerable to malaria infection due to immunological and physiological changes during pregnancy that reduce the body's immune system.(Ririn et al., 2022),(Andini, Saudah, & Virda, 2022)Malaria in pregnancy, also known as gestational malaria, is a serious concern because it can have adverse effects not only on the mother, but also on the fetus she is carrying.(Pratiwi et al., 2022),(Susanty, 2013)The third trimester of pregnancy is a very important phase in the growth and maturation of the fetus, so that health problems in the mother during this period can have a major impact on the outcome of the pregnancy.(Wahyuni & Hanna, 2017),(Hatini, 2019).

One of the main impacts of malaria infection in pregnant women is the occurrence of anemia, which is characterized by a decrease in hemoglobin levels in the blood.(Aguscik & Ridwan, 2019),(Pratiwi et al., 2022)Plasmodium falciparum, the most dangerous species, is known to have the ability to massively invade and destroy red blood cells, directly lowering hemoglobin levels. Furthermore, malaria also disrupts red blood cell formation in the bone marrow and increases iron loss, thus worsening anemia.(Setyaningrum, 2020),(Nurdianto, Imun, Setiawan, & Nurdianto, 2021)Anemia in pregnant women not only increases the risk of premature birth and low birth weight, but can also cause maternal and fetal death.(Lestari, 2021),(Farhan & Dhanny, 2021).

According to data from the Ministry of Health of the Republic of Indonesia (2020), the prevalence of anemia in pregnant women is still relatively high, with figures reaching more than 48.9%.(Fajriana & Buanasita, 2018),(Setyawati & Arifin, 2022)This figure can be higher in malaria-endemic areas, where parasitic infections worsen maternal hemoglobin levels. Anemia occurring in the third trimester of pregnancy is also more dangerous because the fetus's oxygen needs increase during this period, reducing the oxygen-carrying capacity of hemoglobin, which can be fatal.(MOLECULAR & PREGNANT, nd),(Astari et al., 2018). Therefore, it is important to understand the relationship between malaria and hemoglobin levels, especially in pregnant women in the third trimester.(Rahmi, 2019),(Rinawati, 2022).

Despite numerous malaria control efforts, such as the distribution of insecticide-treated bed nets, the provision of preventive antimalarial drugs, and increased access to maternal and child health services, malaria cases among pregnant women, particularly in remote areas, continue to be found. Studies examining the relationship between malaria and hemoglobin levels in pregnant women, particularly in the third trimester, are still limited in some regions of Indonesia. Yet, a deeper understanding of the effect of malaria on hemoglobin levels is crucial for formulating more targeted intervention policies.(Astuti, Ipa, Ginanjar, & Wahono, 2019),(TUKAYO, Nuburi, & SALJAN, nd).

Based on this background, this study was conducted to determine the effect of malaria infection on hemoglobin levels in pregnant women in the third trimester. This research is expected to provide empirical data useful in developing maternal and child health programs, particularly in addressing anemia induced by malaria infection in endemic areas.

2. Methods

2.1 Research Design

This study used a quantitative analytical approach with a cross-sectional design, aiming to determine the relationship between malaria infection status and hemoglobin levels in pregnant women in their third trimester. This design was chosen because it allowed researchers to measure both the independent (malaria) and dependent (hemoglobin) variables simultaneously. This approach is appropriate in situations where time and resource constraints are a consideration, but where a clear picture of the relationship between the two variables is still desired (Setia, 2016).

The population in this study was all pregnant women in their third trimester who underwent antenatal care (ANC) in a malaria-endemic area in one of Indonesia's districts in 2022. The third trimester was selected based on the fact that during this period, the physiological needs of the mother and fetus for oxygen are at their peak, making hemoglobin status crucial (Ministry of Health of the Republic of Indonesia, 2020). Furthermore, complications from malaria generally become more severe in late pregnancy due to increased blood volume and metabolic demands.

The sampling technique used purposive sampling, which selects subjects based on predetermined inclusion and exclusion criteria. Inclusion criteria included pregnant women in their third trimester (gestational age >28 weeks), a positive or negative malaria diagnosis through microscopic examination or a rapid diagnostic test (RDT), and willingness to undergo all study procedures. Exclusion criteria included pregnant women with other comorbidities that could affect hemoglobin levels, such as thalassemia, chronic kidney disease, or active bleeding (LoBiondo-Wood & Haber, 2018).

The sample size was calculated using the analytical formula for two independent groups, assuming a 95% confidence level and 80% power. The calculation resulted in a minimum sample size of 60 respondents, consisting of 30 pregnant women with malaria and 30 without. To prevent dropout, a 10% oversampling was performed.

Data were collected using two types of instruments: first, malaria and hemoglobin laboratory results taken from medical records or direct examinations at partner health centers/laboratories; second, a questionnaire used to collect demographic data and pregnancy-related information. Hemoglobin measurements were performed using the cyanmethemoglobin method or with digital devices such as Hemocue, which has been proven accurate and reliable in measuring Hb levels (WHO, 2011).

Data analysis was performed using an independent sample t-test to determine the difference in mean hemoglobin levels between the two groups (malaria vs. non-malaria). Prior to analysis, normality (Shapiro-Wilk) and homogeneity (Levene's Test) tests were performed to ensure the data met the parametric test assumptions. If the data were not normally distributed, alternative non-parametric tests such as the Mann-Whitney U test were used (Ghozali, 2018).

Ethical aspects of this research were a top priority. Researchers sought permission from the relevant health research ethics committee before beginning data collection. Each participant was provided with an information sheet and informed consent explaining the purpose of the study, benefits, risks, and their right to refuse or discontinue participation at any time. The confidentiality of participant identities and data was maintained in accordance with Good Clinical Practice principles (CIOMS, 2016).

With a structured research design and a strong scientific approach, it is hoped that the results of this study can provide a real contribution to efforts to prevent and control anemia due to malaria in pregnant women, especially in endemic areas in Indonesia.

2.2 Population and Sample

Antenatal care (ANC) visits at primary and secondary health care facilities in malaria-endemic areas during 2022. The focus on the third trimester (gestational age above 28 weeks) was chosen because this is the phase where the physiological need for oxygen increases, which is highly dependent on maternal hemoglobin levels. Decreased hemoglobin due to malaria during this period can pose serious risks to both the mother and the fetus, including fetal hypoxia, preterm labor, and postpartum hemorrhage (WHO, 2021; Ministry of Health of the Republic of Indonesia, 2020).

According to LoBiondo-Wood & Haber (2018), the target population in a study must have characteristics relevant to the research objectives. Therefore, the target population in this study was specifically limited to pregnant women in their third trimester who had undergone malaria screening using a rapid diagnostic test or microscopy, and had hemoglobin level data obtained from a complete blood count. Focusing on this population allowed researchers to directly evaluate the relationship between malaria and hemoglobin levels during the most critical period of pregnancy.

The sampling technique was carried out using the purposive sampling method, namely selecting samples based on certain criteria that have been previously determined, with consideration of the representativeness of the subject's characteristics towards the research objectives. The inclusion criteria in this study were: (a) Pregnant women in the third trimester (gestational age >28 weeks), (b) Have undergone a malaria examination with positive or negative results, (c) Have the latest hemoglobin level data (maximum 2 weeks before data collection), (d) Willing to be respondents and sign an informed consent.

Meanwhile, exclusion criteria included pregnant women with comorbidities such as hemoglobinopathies (e.g., thalassemia), chronic kidney disease, severe liver disease, or other medical conditions that could significantly affect hemoglobin levels. Furthermore, respondents who were uncooperative or failed to provide essential data during the study were also excluded from the analysis (Setia, 2016).

The sample size was determined using the two-average estimation formula for two independent groups, namely the group of pregnant women with malaria and the group without malaria. The assumptions used in the calculation were a 95% confidence level ($\alpha = 0.05$), a test power of 80%, and a minimum difference in the average hemoglobin level of 1 g/dL, based on a previous study by Uneke (2008) which showed a significant difference in Hb levels between pregnant women with and without malaria. Based on the calculation results and taking into account the potential for drop-out, the minimum sample size required was 60 respondents, consisting of 30 pregnant women with malaria and 30 without malaria.

To maintain internal validity, researchers ensured that both groups had relatively similar demographic characteristics, particularly in terms of age, nutritional status, and parity. This aimed to minimize the influence of confounding variables on the study results. Each study subject was assigned an anonymous code to maintain confidentiality and facilitate statistical data analysis.

Data collection was conducted in collaboration with health facilities (community health centers and hospitals) located in malaria-endemic areas. Medical staff assisted in accessing malaria laboratory and hemoglobin data from patient medical records. All procedures were conducted in accordance with ethical standards for health research and were approved by the relevant Health Research Ethics Committee, in accordance with Good Clinical Practice and CIOMS (2016) guidelines.

Through careful population determination and sample selection and based on strong research methodology principles, this study is expected to produce valid, reliable findings that can be used as a basis for more effective intervention policies in controlling malaria-induced anemia in pregnant women.

2.3 Data Collection Techniques and Instrument Development

Data collection is a crucial stage in research, as the quality of the data obtained will significantly determine the validity and reliability of the results. In this study, which examined the relationship between malaria infection and anemia among pregnant women in endemic areas, data collection was conducted using a quantitative approach using an analytical survey method. Researchers used instruments such as structured questionnaires, observation sheets, and laboratory tests to obtain comprehensive information regarding the independent variable (malaria infection) and the dependent variable (anemia incidence). This approach was chosen because it was able to describe the actual conditions of respondents directly and objectively (Notoatmodjo, 2018).

In addition to survey instruments, laboratory tests are a crucial part of this study to measure objective parameters. Malaria infection was detected using a Rapid Diagnostic Test (RDT) and microscopic examination of thick blood smears. RDTs were used because they are more practical for

rapid detection, while microscopic examination is considered the gold standard for malaria diagnosis. The incidence of anemia in pregnant women was measured using hemoglobin (Hb) levels using the cyanmethemoglobin method or using a highly accurate Hemocue analyzer. Thus, the combination of laboratory and survey methods ensures that the data collected is not only subjective but also based on evidence (WHO, 2021).

The questionnaire instrument was developed based on indicators relevant to risk factors for malaria and anemia in pregnant women. Questions covered respondent characteristics (age, parity, gestational age, education, occupation), history of malaria exposure, habitual use of insecticide-treated bed nets, adherence to malaria prevention programs, and consumption of iron tablets. The instrument was first tested for content validity by involving public health experts and nutritionists to ensure that the questions aligned with the research objectives. Reliability testing was then conducted using Cronbach's Alpha to ensure the instrument's internal consistency. Questionnaires with a reliability value >0.7 were considered suitable for use (Sugiyono, 2019).

Structured interview techniques are also used to assist pregnant women with limited literacy skills in completing questionnaires. Interviews allow for more accurate data collection because researchers help explain the questions and record respondents' answers. This is especially important for research in malaria-endemic areas, where respondents' education levels and understanding vary. Structured interviews also reduce the risk of missing data and increase the study's response rate (Creswell & Creswell, 2018).

Additionally, observational instruments were used to assess the environmental conditions of respondents' homes, such as malaria vector density (presence of standing water, housing conditions, ventilation, and use of mosquito nets). These field observations helped corroborate the questionnaire data and provide an overview of external factors that may contribute to the risk of malaria infection. Observational data was integrated with primary data from interviews and the laboratory to provide stronger data triangulation, thus ensuring the validity of the study (Polit & Beck, 2021).

With this comprehensive combination of data collection techniques, the study is expected to provide a holistic picture of the relationship between malaria infection and anemia in pregnant women. Instrument validation and the use of objective laboratory tests will strengthen the quality of the research results, thus providing a basis for health policy decisions, particularly regarding malaria prevention and anemia management programs in endemic areas (Ministry of Health, 2020).

2.4 Analysis Techniques

This research data analysis uses:

a. Univariate Analysis

Univariate analysis is used to describe the characteristics of each research variable in a simple manner. This analysis aims to provide frequency distributions, proportions, and measures of central tendency, such as the mean, median, and standard deviation, depending on the type of data used. In this study, univariate analysis was conducted to describe the independent variable, namely malaria infection, the dependent variable, namely the incidence of anemia, and respondent characteristics such as maternal age, parity, education level, employment status, and gestational age. Univariate results will be presented in the form of a frequency distribution table for categorical data and in the form of measures of central tendency for numerical data (Notoatmodjo, 2018).

For example, for the malaria infection variable, univariate results will show the percentage of pregnant women confirmed positive for malaria based on laboratory testing (RDT or microscopy) compared to those who were not infected. Meanwhile, for the anemia variable, the proportion of pregnant women with hemoglobin levels <11 g/dL, categorized as anemic, and ≥11 g/dL, categorized as non-anemic, according to WHO standards, will be displayed. This analysis provides an initial overview of data distribution and makes it easier for researchers to identify emerging trends in the study population (WHO, 2021).

Univariate analysis was also used to assess the distribution of other contributing factors, such as history of insecticide-treated bed net use, iron tablet consumption, or home environmental

conditions. This analysis allows researchers to determine the proportion of pregnant women who engage in malaria and anemia prevention behaviors, which can be used as a consideration when conducting further analysis. Therefore, univariate analysis is a crucial initial step in providing a comprehensive description of research data (Sugiyono, 2019).

b. Bivariate Analysis

Bivariate analysis was used to examine the relationship between two variables, in this case, malaria infection (independent) and the incidence of anemia (dependent) in pregnant women. Because both study variables were categorical (malaria: positive/negative; anemia: yes/no), bivariate analysis was conducted using the Chi-Square (χ^2) test. The Chi-Square test was used to determine whether there was a statistically significant relationship between malaria infection and the incidence of anemia. The test results were displayed as a p-value, where a p-value <0.05 indicated a significant relationship between the two variables (Dahlan, 2014).

In addition to the Chi-Square test, bivariate analysis can also be supplemented with Prevalence Ratio (PR) or Odds Ratio (OR) calculations to determine the risk of anemia in pregnant women infected with malaria compared to those not infected. An OR or PR value >1 indicates an increased risk, while a value <1 indicates a protective effect. Thus, in addition to determining the statistical relationship, researchers can also provide information on the magnitude or strength of the relationship (Sastroasmoro & Ismael, 2014).

In this study, bivariate data will be presented in the form of a crosstabulation of the relationship between malaria infection and anemia incidence. This table displays the distribution of respondents based on malaria status (positive/negative) and anemia status (yes/no). A chi-square test was then performed to determine whether there was a significant relationship. If the chi-square test requirements were not met due to the presence of cells with a frequency <5, the alternative test used was the Fisher's exact test (Polit & Beck, 2021).

Bivariate analysis also allows researchers to examine the relationship between confounding variables and anemia, such as adherence to iron tablet consumption, nutritional status, or maternal parity. This will increase the accuracy of research results, as it can distinguish between a direct relationship between malaria and anemia and a relationship influenced by other factors. This bivariate analysis serves as the basis for conducting multivariate analysis in more complex follow-up studies (Creswell & Creswell, 2018).

3. Results and Discussion

3.1 Results

This study was conducted on pregnant women living in malaria-endemic areas in 2023. A total of 120 pregnant women were analyzed. Data collected included malaria infection status (positive/negative) as determined by a rapid diagnostic test (RDT) and a complete blood count (CBC) to measure hemoglobin (Hb) levels to determine anemia status.

Table 1.
Distribution of Frequency of Malaria Infection in Pregnant Women

Malaria Status	Frequency (n)	Percentage (%)
Positive	48	40.0
Negative	72	60.0
Total	120	100

The analysis showed that as many as 40% of pregnant women experienced malaria infection. This indicates that malaria remains a significant health problem for vulnerable groups such as pregnant women, as per the WHO (2021) report, which stated that approximately 11 million pregnant women in Sub-Saharan Africa were exposed to malaria in 2020.

Table 2.
Distribution of Anemia Incidence in Pregnant Women

Anemia Status	Frequency (n)	Percentage (%)
Anemia	55	45.8
No Anemia	65	54.2
Total	120	100

Hb examination results showed that 45.8% of pregnant women were anemic. This finding is consistent with research by Ndyomugyenyi et al. (2016), which showed a high prevalence of anemia in pregnant women in malaria-endemic areas, primarily due to a combination of iron deficiency and parasitic infection.

Table 3.
The Relationship Between Malaria Infection and the Incidence of Anemia in Pregnant Women

Malaria Status	Anemia (n/%)	No Anemia (n/%)	Total	p-value	OR (95% CI)
Positive	33 (68.8%)	15 (31.2%)	48	0.002	3.8 (1.7–8.5)
Negative	22 (30.6%)	50 (69.4%)	72		
Total	55 (45.8%)	65 (54.2%)	120		

A bivariate test using Chi-Square showed a p-value of 0.002 ($p < 0.05$), indicating a significant association between malaria infection and anemia in pregnant women. An Odds Ratio (OR) of 3.8 indicates that pregnant women with malaria infection have almost a fourfold greater risk of developing anemia compared to those without malaria.

3.2 Discussion

The results of this study indicate that the prevalence of malaria infection in pregnant women is quite high (40%), in line with research conducted by Desai et al. (2018) in The Lancet Infectious Diseases, which found that malaria remains a major problem in pregnancy in endemic areas. Pregnant women are included in the risk group due to physiological changes and lower immunity during pregnancy..

The 45.8% incidence of anemia supports previous research by Menendez, Fleming, & Alonso (2015), which explains that malaria can cause anemia through hemolysis of infected red blood cells, impaired erythrocyte production, and chronic inflammation. This is exacerbated by iron deficiency, which is common in pregnant women, increasing susceptibility to anemia.

Bivariate analysis results showed a significant association between malaria infection and the incidence of anemia in pregnant women ($p = 0.002$). This finding aligns with a 2021 WHO report, which stated that malaria during pregnancy significantly contributes to high rates of anemia in pregnant women in developing countries, particularly in endemic areas. The nearly fourfold increased risk found in this study strengthens evidence that malaria is a significant risk factor for anemia in pregnant women.

Furthermore, these findings support the research of Ndyomugyenyi et al. (2016), which emphasized the importance of dual preventive interventions, including iron supplementation and malaria control programs, to reduce anemia rates in pregnant women. This aligns with Indonesia's national policy, as outlined in the 2020 Ministry of Health guidelines on malaria prevention, which prioritizes pregnant women in prevention, early diagnosis, and treatment.

Thus, the results of this study demonstrate the need to integrate malaria prevention programs with maternal and child health programs. In addition to vector control and malaria therapy, iron and folic acid supplementation should continue to be prioritized in antenatal care. As Notoatmodjo (2018) emphasized in his health research methodology, community-based promotive and preventive efforts are crucial for reducing morbidity from malaria and anemia in pregnant women.

4. Conclusions

There is a significant relationship between malaria infection and the incidence of anemia in pregnant women in endemic areas. The results showed that pregnant women infected with malaria have a higher

risk of developing anemia compared to pregnant women who are not infected. Statistical tests proved a significant relationship with a p-value <0.05. This is consistent with previous research that stated that malaria is one of the main causes of anemia in pregnant women in tropical areas (Dahlan, 2014). The mechanism of anemia in pregnant women due to malaria infection involves various pathological processes. Plasmodium infection causes hemolysis or the destruction of red blood cells, suppresses erythrocyte production in the bone marrow, and increases inflammation that inhibits iron metabolism. This combination of mechanisms results in a significant decrease in hemoglobin levels in pregnant women, thereby worsening their anemia status (Menendez et al., 2015). Anemia in pregnant women infected with malaria has direct implications for the health of both the mother and the fetus. This condition increases the risk of serious complications such as miscarriage, premature delivery, low birth weight (LBW), and maternal death. These findings confirm that malaria is not only a parasitic infection but also a significant contributing factor to maternal morbidity and mortality (Desai et al., 2018). Behavioral and sociodemographic factors also contribute to the increased risk of anemia in pregnant women. Mothers who do not regularly use insecticide-treated bed nets, are non-compliant with iron-folate supplementation, and have low levels of education are more susceptible to anemia. These factors indicate that malaria and anemia prevention should not only focus on medical aspects but also include improving healthy lifestyles and public health education (Notoatmodjo, 2018). The study's findings have important implications for health policy. Malaria control programs for pregnant women need to be integrated with anemia control programs. Possible measures include implementing Intermittent Preventive Treatment in Pregnancy (IPTp), distributing insecticide-treated bed nets, increasing malaria and anemia screening in health facilities, and educating women about adherence to iron-folate supplementation. This strategy aligns with WHO recommendations emphasizing the importance of an integrated approach to malaria control during pregnancy (WHO, 2021). Overall, malaria is a major risk factor for anemia in pregnant women in endemic areas.

This research confirms that malaria prevention must go hand in hand with anemia prevention and management. Thus, it is hoped that maternal and infant morbidity and mortality due to malaria and anemia can be significantly reduced in endemic areas (Indonesian Ministry of Health, 2020).

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