Detection of Malaria Antigen by Rapid Diagnostic Method in Student STIKes Guna Bangsa Yogyakarta Origin East Nusa Tenggara

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Abstract. *Malaria*, caused by Plasmodium protozoa, affects 300-500 million annually, resulting in 1.5 to 2.7 million deaths worldwide. Particularly prevalent in East Nusa Tenggara, ranking third in malaria morbidity after Papua and West Papua One of the processes of transmitting malaria is through blood transfusions. Researchers see the need for malaria antigen testing to prevent the spread of malaria, especially to students from East Nusa Tenggara. This research aims to determine the proportion of malaria incidence among STIKes Guna Bangsa students from the East Nusa Tenggara area. The type of research used was descriptive observational with a cross-sectional design. The total sample was 36 students with malaria antigen screening using the Rapid Diagnostic Test (RDT) method (Standareagen diagnostic kit for Malaria Pf/Pv antigen), analyzing risk factors for malaria incidence and measuring hemoglobin levels. Generally, malaria is characterized by low hemoglobin levels. The results of this research were that 100% of the samples were non-reactive to malaria with normal hemoglobin levels, so it could be concluded that there was no incidence of malaria in the students' blood analysis samples from either the RDT or risk factors.

1 .Introduction

Malaria is an acute infectious disease caused by protozoa from the genus Plasmodium. The malaria parasite is transmitted through the bite of the female Anopheles mosquito. Generally, four species cause malaria that infect humans, namely *P. falciparum*, *P. vivax*, *P. ovale*, and *P. Malaria*. Another research reported that another plasmodium species infects humans, namely *Plasmodium knowlesi*, which has been known to infect humans since 1960 [1]. Based on Indonesia's health profile in 2020, malaria-endemic areas in Indonesia include Papua, West Papua, East Nusa Tenggara, East Kalimantan, and Maluku. East Nusa Tenggara is the province with the third highest malaria morbidity rate after Papua and West Papua, with a malaria morbidity rate of 2.76 per 1000 population. This figure has increased from the previous year, 2.37 per 1000 population [2].

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Transmission of malaria through blood transfusions poses a threat to the safety of blood products and risks increasing malaria transmission. Several malaria-endemic countries have reported blood donor surveillance results that were positive for malaria, such as Africa and several countries in Asia. There have been no reports of malaria transmission through blood transfusions in Indonesia. Malaria screening of donor blood in Indonesia only focuses on endemic areas [3].

Cases of malaria transmission through blood transfusions are rare, but can cause serious reactions in the body of recipients or patients who are not immune (do not have antibodies) to malaria [4]. The number of malaria transmission through blood transfusions was recorded at 97 cases until 2011 [3]. The most common species transmitted through blood transfusions are *P. falciparum*, *P. malariae* and *P. vivax*. Plasmodium species can survive for 20 days in blood at a temperature of 2oC – 6oC, which stores Packed Red Cells (PRC) blood products [5].

Donors from endemic areas with a history of malaria infection have formed antibodies in their bodies. However, some malaria species can survive in the body even though the sufferer has been treated or declared cured. When someone from a malaria-endemic area travels to a non-endemic area, problems will arise if there is a relapse because malaria treatment is rarely found in non-endemic areas. A person infected with malaria in a non-endemic area will experience difficulties in treatment and may be diagnosed with another disease. In addition, asymptomatic malaria carriers have the potential to be a source of malaria transmission through blood transfusions, considering that the life cycle of Plasmodium can last several weeks in the blood [4].

Yogyakarta is one of the provinces in Indonesia called a student city because there are many universities, and it is a study destination for students from various regions in Indonesia, both from non-endemic areas and malaria-endemic areas. STIKes Guna Bangsa is one of the universities with many students from malaria-endemic areas. Based on the student data obtained, 78 students from the STIKES Guna Bangsa Yogyakarta Diploma program came from the East Nusa Tenggara area. Based on this data, 36 students will be selected for malaria antigen screening. Considering that STIKes Guna Bangsa students often involve themselves in blood donation activities, it is very likely that students who come from endemic areas can be a source of malaria transmission, so in this research, Malaria Antigen detection will be carried out in students from East Nusa Tenggara to safeguard the transfusion process and also used as early detection/initial examination so that it can increase the safety of blood products for the transfusion process.

2 .Methodology

This research uses descriptive observational research with a cross-sectional design. Samples were taken from peripheral blood and examined using the RDT method (Standareagen diagnostic kit for Malaria Pf/Pv antigen) to determine the presence or absence of malaria antigens in STIKes Guna Bangsa Yogyakarta students who came from the East Nusa Tenggara area.

2.1 Sampels

The research sample was 36 STIKes Guna Bangsa Yogyakarta students from East Nusa Tenggara who were willing to be respondents and had signed an informed consent. The research sample was taken based on the sample size calculation using the Slovin formula as Equation (1).

$$n = \frac{N}{(1+Ne^2)} \quad (1)$$

Information :
n : Sample size/sample size
N : Population
e^2 : *margin of eror*

The criteria for this research are as follows:
a. Inclusion Criteria
1. STIKes Guna Bangsa students for the 2020 and 2021 classes
2. STIKes Guna Bangsa students who come from the East Nusa Tenggara area
3. Students willing to be respondents have filled out the informed consent form.
b. Exclusion Criteria
1. STIKes Guna Bangsa students who are undergoing malaria treatment

2.2 Peripheral Blood Collection

The pricked finger is pressed until blood comes out, and the first blood is wiped using dry cotton. A hemoglobin check is carried out using automatic Hb, where the next blood that comes out is put into a strip until it fills the entire strip. Then, wait until the calculation is complete and the results are read. After the hemoglobin examination, the malaria examination continues. Prepare a dropper pipette, then take blood using it. The blood in the pipette is dropped into the rapid test tool as much as one drop. Next, two drops of buffer solution were added. Wait 15 to 20 minutes until the reaction is complete. Observations and interpretation of the results were carried out.

2.3 Data Analysis

Research data were collected and analyzed using the SPSS program, presented in tabular form as percentage data to determine the proportion of malaria cases detected among students of STIKes Guna Bangsa Yogyakarta from the East Nusa Tenggara area.

3 .Result and Discussion

The results of the malaria antigen examination and the questionnaire data obtained were then analyzed using the SPSS program. The results obtained are as follows:

3.1 Respondent Characteristics

The malaria antigen screening research was attended by 36 respondents who were students of STIKes Guna Bangsa Yogyakarta from East Nusa Tenggara with gender dominated by respondents with female gender amounting to 63.9% and males amounting to 36.1%, with the most vulnerable age of the respondents varies from 18 to 22 years.

3.2 Test Examination Results using the Rapid Diagnostic Test Method

The malaria antigen screening examination in this study used the Rapid Diagnostic Test (RDT) method with the Rapid Cassette Standard Reagent One Step Malaria pf (HRP II)/ pv(pLDH) Antigen Detection Test Device (Whole Blood) type. Examination using RDT is easy, fast and relatively simple. Data from malaria screening using RDT shows that the percentage of malaria incidence among STIKes Guna Bangsa Yogyakarta students from the

East Nusa Tenggara area is 0% (Table 1). This shows that no malaria antigen was detected in the students' blood samples.

Table 1. Malaria examination results using the RDT method

RDT Examination Results	Frequency	Percentage%
Reactive	0	0%
Non-reactive	36	100%

Based on data from malaria antigen screening results using the RDT method in Table 1, the results were 100% non-reactive to malaria antigens. The percentage of malaria incidence among STIKes Guna Bangsa Yogyakarta students is 0%. The true negative results in this study could be caused by parasitic factors that were not present in the respondents' blood samples, as well as the good sensitivity and specificity of the equipment in the examination. Respondents' behavior when they were in their area of origin was also one of the factors that did not show malaria incidence in this study. Likely, respondents always use personal protection from mosquitoes by using mosquito repellent and wearing mosquito nets when sleeping at night to avoid mosquito bites and are not infected with malaria.

Apart from true negative results in this study, there is the possibility of false negatives. This can be caused by several factors, including the small number of parasites in blood samples, as stated by Alydrus *et al.*, [6], in research entitled "Comparison of microscopic methods and rapid diagnostic tests for Plasmodium detection in malaria sufferers in Ambon city", stated that examinations using RDT were only able to read parasite densities of 40-100 parasites per microliter of blood. So, if the parasite density in the blood sample is low or less than 40 parasites per microliter of blood, the parasites will not be detected by RDT.

Another factor influencing non-reactive examination results is the deletion of the HRP-2 antigen or the mutation of the HRP-2 antigen to HRP-3. If an antigen mutation occurs in the blood sample, it cannot be detected by the Pf-HRP2 RDT. The Pf-HRP3 antigen produced by the parasite codes for alanine and histidine-rich amino acids, similar to the Pf-HRP2 antigen. However, the difference lies in the amino acids. These genetic variations can affect the detection of P. falciparum parasites [7].

Not obtaining reactive results against malaria can also be caused by equipment limitations. The RDT used in this study could only detect *Plasmodium falciparum* and *Plasmodium vivax* species. If the blood sample is infected with other malaria parasites (*P. ovale*, *P. malariae*, and *P. knowlessi*) it cannot be detected on the rapid diagnostic test tool.

3.3 Hemoglobin Level Examination Results

In this study, hemoglobin levels were measured using easy touch type automatic hemoglobin. The results of the examination of hemoglobin levels are shown in Table 2.

Table 2. Results of Hemoglobin Levels

Hemoglobin levels	Frequency	Percentage%
Low (<12,5g/dL)	7	19,4%
Normal (12,5-17 g/dL)	26	72,2%
High (>17 g/dL)	3	8,3%
Total	**36**	**100%**

Table 2 shows that 72.2% had normal hemoglobin levels, followed by 19.4% low hemoglobin levels and 8.3% high hemoglobin levels. A percentage of 19.4% of respondents with low hemoglobin levels could be said to have anemia. As stated by Panjaitan *et al.*, [8],

the decrease in hemoglobin levels in malaria infection is due to excessive destruction of red blood cells by malaria parasites. Infection of red blood cells occurs in the erythrocytic cycle (the parasite life cycle that occurs in blood cells). The release of *Plasmodium merozoites* will then enter the bloodstream and infect erythrocytes. The Plasmodium parasite causes anemia as a result of hyperhemolysis of red blood cells or excessive destruction of red blood cells and reduces erythropoiesis.

The results of examining hemoglobin levels in this study showed that 19.4% of respondents had low hemoglobin levels, in this condition there was an incidence of anemia. However, the incidence of anemia was not influenced by the incidence of reactive malaria. Based on research by Suryadi *et al.*, [9] examined the relationship between the types of Plasmodium falciparum and Plasmodium vivax with the incidence of anemia in malaria patients at the Ratu Aji Putri Botung Regional Hospital, Penajam Paser Utara, stated that statistically there was no relationship between the type of Plasmodium falciparum and Plasmodium vivax with the incidence of anemia with a value of $p = 0.391$ ($p > 0.05$).

The results of this study also found no association between low hemoglobin levels in 19.4% of respondents and the incidence of malaria as seen in Table 2, which shows that 100% of blood samples from STIKes Guna Bangsa Yogyakarta students from the East Nusa Tenggara region were non-reactive to malaria. However, they were due to several other factors that influenced the decrease in hemoglobin levels as stated in Saputro and Junaidi [10], including lack of activity, unhealthy eating patterns, and behavior, unhealthy living such as smoking. In the remainder of the study, no further review was carried out regarding the causes of low hemoglobin levels.

3.4 Risk Factor Analysis

This study also investigated the risk factors contributing to malaria incidence to complement the malaria antigen examination findings. Researchers obtained the risk factors through questionnaires distributed to respondents before collecting research samples. Respondents were then asked to answer several questions regarding the risk factors presented in the provided questionnaire. The results and analysis of risk factors obtained are as follows:

3.4.1 Percentage of Malaria History

A history of malaria serves as a risk factor for subsequent malaria infections. Individuals who have experienced or have a history of malaria, including multiple Plasmodium infections such as P. vivax, are likely to experience relapse or recurrence within a certain period. Data regarding the incidence of malaria history are presented in Table 3.

Table 3. Percentage of Malaria History

Information (History of Malaria)	Frequency	Percentage%
Yes No	6 30	16,7% 83,3%
Total	36	100%

Data in Table 3 shows that 16.7% of respondents had experienced or had a previous history of malaria. A person with a previous history of malaria is likely to experience a relapse or recurrence, which can occur at any time. According to Karyus and Rahayu [11], relapse occurs in *P. vivax* at most 2 – 5 years. Recurrence in *Plasmodium vivax* is caused by the presence of a dormant hypnozoite phase in liver cells, making it possible for sufferers to experience a secondary malaria attack.

This study revealed that 16.7% of respondents with a prior history of malaria did not experience any further malaria incidents, as indicated in Table 3. This observation was linked to the acquisition of rapid diagnostic test results showing 100% non-reactivity to malaria incidents. The absence of malaria incidents in 16.7% of respondents was attributed to the absence of the malaria parasite in their bloodstreams. This phenomenon may be associated with the behavior of respondents who consistently undergo malaria treatment.

According to research by Shafira and Kristanti [12], stated that imperfect malaria treatment can cause drug resistance, allowing the parasite to survive even though the drug dose according to the treatment standard has been given. Drug resistance will have an impact on the occurrence of relapse as a result of incomplete elimination of parasites due to inadequate treatment processes.

3.4.2 Percentage Returning to Area of Origin

The activity of returning to the area of origin, which is a malaria-endemic area, can be a risk factor for the incident. Referring to blood donation activities, the informed consent also contains questions about the donor's history of travelling to malaria-endemic areas before donating blood. Donors who have traveled to malaria-endemic areas for a certain duration are temporarily deferred to mitigate the risk of malaria transmission through blood transfusions, as frequent returns or visits to malaria-endemic areas increase the likelihood of contact with malaria vectors. The percentage of time spent returning to the area of origin is presented in Table 4.

Table 4. Percentage returning to area of origin

Information	Frequency	Percentage%
The last time the individual returned to their area of origin within the last year.		
<1 month	3	8,3%
>1 month	33	91,7%
The frequency of returning to the area of origin within the last year.		
1-2 time	36	100%
>2 time	0	0%
Total	36	100%

According to the percentage data on returning to their area of origin presented in Table 4, it was observed that 8.3% of respondents returned for less than 1 month, while the remaining 91.7% returned for more than 1 month. Additionally, all respondents returned to their area of origin at least once in the last year, with a frequency of 1-2 times. Returning to one's area of origin is common among respondents, typically occurring at the end of each semester during the study period. This behavior can serve as a risk factor for malaria incidence. Prolonged residence in the area of origin exposes respondents to potential contact with Anopheles mosquitoes, particularly if they engage in outdoor activities at night without using personal protective measures such as mosquito repellent or sleeping under mosquito nets. Additionally, mosquito breeding sites in the vicinity, as noted by Isnaeni *et al.* [13], underscores how both behavioral and environmental factors among respondents contribute to the risk of malaria transmission.

In this study, 100% of respondents returned to their area of origin 1-2 times, with 8.3% returning for less than one month and 91.7% for more than one month. Notably, no incidents of malaria were reported. These findings regarding the history of respondents' return to their area of origin suggest that the absence of malaria incidents could be attributed to the respondent's brief stays in the area and their cautious behavior towards malaria infection, thereby reducing the likelihood of contact with the vector, namely the Anopheles mosquito.

Furthermore, no further analysis or review was conducted regarding the duration of time respondents spent living in their area of origin.

3.4.3 Respondent Behavior in the Area of Origin

Another risk factor that influences the incidence of malaria is the respondent's behavior when they are in their area of origin, such as leaving the house at night, using mosquito nets when sleeping, and using mosquito repellent. Data regarding respondents' behavior in their area of origin is shown in Table 5.

Table 5. Distribution of Respondents Based on Behavior in Area of Origin

Information	Frequency	Percentage%
Activities outside the house at night		
Yes	23	63,9%
No	13	36,1%
Use of mosquito nets when sleeping at night		
Yes	22	61,1%
No	14	38,9%
Use of anti-mosquito (insecticide, repellent)		
Yes	16	44,4%
No	20	55,6%
Total	36	100%

An overview of respondents' behavior in their area of origin is provided in Table 5. The most frequently reported behavior among respondents is leaving the house at night, with 63.9% engaging in this activity. Conversely, the least reported behavior is the use of mosquito repellent, with only 44.4% of respondents indicating its usage.

Outdoor activities at night are associated with the incidence of malaria because the Anopheles mosquito, known for its nocturnal activity, primarily bites outside homes during peak hours, typically between 00:00:00 and 01:00 [14]. In this study, 63.9% of respondents were observed to engage in outdoor activities at night in their area of origin. Research conducted by Papilaya *et al.* [15] concluded a correlation between nighttime activities and malaria incidence. However, this study did not establish a direct link between leaving the house at night and malaria incidence among students from STIKes Guna Yogyakarta originating from East Nusa Tenggara.

In this study, 61.1% of respondents reported the habit of using mosquito nets when sleeping at night. This practice aims to prevent mosquito bites, as mosquito nets are effective in minimizing contact with mosquitoes. Using insecticide-treated mosquito nets is considered the most effective method because mosquitoes typically bite at night when people are asleep. According to Mustafa [16], insecticide-treated mosquito nets reduce human contact with mosquitoes by either killing them upon contact with the net or repelling them.

Based on research conducted by Hamzah [17], the use of mosquito nets is related to the incidence of malaria, one of the factors being the use of mosquito nets that do not meet the standards of Insecticide Treated Mosquito Nets (ITNs) or nets that do not contain insecticides, thereby allowing contact with Anopheles mosquitoes. The frequency of respondents who did not use mosquito nets when sleeping at night in this study was 38.9%, where there was no incidence of malaria, so it cannot be concluded between the use of mosquito nets and the incidence of malaria.

Several ways that can be done to reduce and prevent malaria include using anti-mosquito medication. The types of anti-mosquito medicines that are widely circulated in the

community are mosquito coils, mosquito sprays, electric mosquito coils (Electric) and mosquito repellent substances (Repllant) [18]. The habit of respondents using mosquito repellent while in their area of origin in this study was carried out by 44.4%, and there were no cases of malaria found in the other 55.6% of respondents who did not use mosquito repellent.

3.4.4 Percentage of Mosquito Breeding Sites

The breeding place for Anopheles mosquitoes is standing water. Stagnant water around the house, such as ponds, rivers and rice fields, can become a breeding place for mosquitoes. Data regarding the percentage of mosquito breeding sites is presented in Table 6.

Table 6. Mosquito breeding places

Information	Frequency	Percentage%
Yes	12	33,7
No	24	66,3
Total	**36**	**100%**

According to the data presented in Table 6, 33.7% of respondents reported having mosquito breeding sites. These breeding sites, such as ponds, rivers, or stagnant puddles of water, serve as a significant source of increased mosquito populations around households. Stagnant water is particularly attractive to mosquitoes for laying eggs, thereby contributing to the proliferation of mosquitoes. This increase in mosquito population poses a risk factor for malaria transmission, heightening the likelihood of mosquito-human contact.

According to studies by Asrul and Kader [19], the proportion of households with mosquito breeding sites and individuals residing near such breeding grounds face twice the risk of contracting malaria compared to those living in environments without such breeding sites. However, in contrast to these findings, this study observed that the presence of mosquito breeding sites did not correlate with the incidence of malaria in 33.7% of respondents.

Based on this research, it can be concluded that the incidence of malaria in STIKes Guna Bangsa Yogyakarta students from the East Nusa Tenggara region is 0% or 100% non-reactive, where there is no incidence of malaria either in the examination using RDT or risk factor analysis, and there are no malaria parasites. (Plasmodium falcivarum and Plasmodium vivax) using the RDT method on blood samples from STIKes Guna Bangsa Yogyakarta students from the East Nusa Tenggara area. a 100% non-reactive result certainly does not eliminate the possibility of transmitting malaria through blood transfusions, so further research is needed

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