Hematological Parameters Variations Among Patients With Uncomplicated *Plasmodium falciparum* Infection in Cambodia

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### Abstract

**Introduction:** The prevalence of anemia among patients with malaria is very common from subsequent erythrocytes destruction and should be managed most appropriately. This study aimed to explore the changes in hematological parameters and their underlying influence among people with uncomplicated *Plasmodium falciparum* infection.

**Methods:** A cross-sectional study was conducted among uncomplicated malaria patients infected by *P. falciparum* on community-based active screening days in one of the highest malaria hot-spot areas of northwestern Cambodia. Descriptive statistics, and student t-tests were used to analyze the data.

**Results:** Among 103 malaria blood samples, the results showed that most participants had thrombocytopenia (84.5%). More than one-half of the participants presented normal levels of the following hematological parameters: red blood cells, hemoglobin, hematocrit, mean corpuscular volume (MCV) and red cell distribution width (RDW). A significant correlation was noted between parasite counts and three body mass index (BMI) groups (*P* = 0.047). Next, a strong association was also seen between parasite counts and body temperature (*P* = 0.001). Statistically significant differences in parasite count were observed across three levels of neutrophil (*P* = 0.005), lymphocyte (*P* = 0.001), eosinophil (*P* < 0.001), absolute lymphocyte (*P* = 0.001) and absolute eosinophil (*P* < 0.001) counts.

**Conclusion:** The results of this study revealed the significant role of hematological parameters in predicting the presence of malaria infection, parasite density, and forecasting adverse consequences of malaria, together with the underlying risk factors.

**Keywords:** Hematology, Parasites, Patients, *Plasmodium falciparum*, Cambodia

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### Citation

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**Introduction**

Malaria, a life-threatening disease that has remained at an endemic level, is caused mainly by *Plasmodium falciparum*, transmitted by the female *Anopheles* mosquitoes.¹ Although malaria is a preventable and curable disease, it continues to constitute a major health problem in several countries. According to 2019 data endorsed by the World Health Organization (WHO), the malaria burden has shown a gradually declining trend, with an 38% reduction in mortality compared with 2010.¹² However, as those data were compiled mainly from public sectors, they might have failed to determine the actual burden of the disease especially in the developing countries.

In 2019, the estimated population of Cambodians living in malaria-endemic areas was 11.3 million, around 70% of the total population, and 32,197 were confirmed as having contracted malaria.² About 20% of the confirmed patients were infected by *P. falciparum*.² Moreover, 46 deaths in 2012 and one death in 2017 were attributed to consequences of adverse complications of malaria, including severe and irreversible anemia.² Generally, populations at risk for malaria are those residing less than two kilometers from forested areas.³ Presently, malaria is detected and treated free-of-charge by all public health departments as well as various nongovernmental organizations’ projects.⁴ Assessing febrile patients, even those with nonspecific signs and symptoms, is important to differentiate between malaria and other febrile illnesses in tropical countries.⁵ To diagnose malaria,
especially at the field level, two kinds of diagnostic methods are widely used. First, rapid diagnostic tests (RDTs) can confirm the disease within 15 minutes with >90% sensitivity and specificity. The second is gold-standard microscopy, which can detect detailed parasite counts and treatment response, in addition to species identification, using patients’ capillary blood. However, both methods have limitations regarding the accuracy, as the quality of results depends on multi-disciplinary factors. The polymerase chain reaction (PCR) method can solve these issues, but this presents challenges in developing countries as it might be costly with poor accessibility, especially in remote regions.

On the other hand, as *P. falciparum* can transform to severe and complicated conditions even within hours or days, proper treatment should be delivered as soon as possible after disease confirmation. Unfortunately, malaria-suspected patients present extensive delays in seeking care, which promotes the occurrence of severe forms of the disease. The prevalence of anemia among patients with malaria is very common from subsequent erythrocytes destruction and should be managed most appropriately. As the functions of hematological parameters are very important to identify signs of anemia, liver and renal functions among patients with malaria to confirm signs of severity and forecasting of changes in hematological parameters becomes essential to undertake immediate management and prevent major hematological complications. The study aimed to explore the changes in hematological parameters and their influences among people with uncomplicated *P. falciparum*-infection.

**Methods**

**Study Design**

This study was a cross-sectional design, combing quantitative methods to determine the correlations between the parasite counts and hematological parameters among uncomplicated malaria patients with *P. falciparum* infection diagnosed by microscopy in one of the high malaria endemic areas in Cambodia.

**Study Site and Sample**

The study was conducted in northwestern Cambodia in 2012. Northwestern Cambodia was the study site due to its high malaria endemicity and the catchment area of the Armed Forces Research Institute of Medical Sciences (AFRIMS). The two sites are purposely selected as the study areas – (a) Ta Sanh Commune, Battambang province and Battambang Referral hospital (8 subjects), (b) Alongveng District, Oudarmeanchey province and surrounding area along the Cambodian-Thai border, determined to have a high incidence of malaria infection (it provided 95 subjects).

**Target Population**

The targeted population was uncomplicated malaria patients with *P. falciparum* infection confirmed by the microscopy.

**Sample Size Determination**

To validate the required sample size for a prevalence study, the following infinite population proportion formula was applied.

$$n = \frac{Z^2 \cdot p(1-p)}{d^2}$$

where $n =$ desired sample size = 101

$p =$ malaria prevalence in the selected study site = 0.07

d = a 5% maximum tolerated error = 0.05

$Z =$ 95% confidence level = 1.96

**Study Subjects**

A total of 103 patients with malaria were confirmed and eligible to participate in the study through convenient sampling under strict screening by inclusion and exclusion criteria. The inclusion criteria consisted of adults aged 18 to 70 years, who submitted informed consent and were infected with uncomplicated *P. falciparum* malaria, with a parasite density of 1000 to 200 000 per microliter, as confirmed by blood smear. The exclusion criteria consisted of patients with severe malaria infection who were found to have a severe glucose-6-phosphate dehydrogenase deficiency at screening, patients who had used any anti-malaria drug during the preceding 14 days, or who were pregnant or breastfeeding.

**Study Tools and Data Collection**

The respective community leaders had been notified two days ahead, to recruit people and disseminate information about the mobile clinic. All those at risk of malaria including patients with fever and who had resided in a forested area were informed to have a blood test to avoid any missing individual. On a predetermined malaria screening day, every individual who came to the mobile clinic was examined using a blood test. A total of four clinics (once monthly) were organized during the malaria peak rainy season (July to October).

In addition, the body weight and height of each participant were measured using a weight and height platform scale. Body temperature was also taken using a tympanic thermometer. The presence and count of malaria parasites were confirmed by certified lab technicians who had had experience reading more than 2000 blood slides under the microscope before joining this study. The blood samples used for the study were taken from the peripheral blood, and hematological parameters were measured using an automatic hematology analyzer.

**Measurement and Key Variables**

Hematological parameters were the key variables in this study that came from the blood samples used for study taken from the peripheral blood analyzed by lab technician specialists using an auto hematology analyzer. Independent variables including parasites confirmed and counts, i.e., the parasites confirmed and count by the lab technician specialists, who had experience reading 100 blood slides before joining this study. The peripheral blood samples and body temperature, i.e., the body temperature was included in the vital signs, were collected by the study nurse. They used a tympanic thermometer to test the body temperature. Height and weight, i.e., all participants in the sample had their body weight and height measured using a weight and height platform scale.
**Data Management and Analyses**

Complete data for each patient were recorded separately and stored in a password-protected computer. After ensuring data quality, descriptive statistics were used to describe each variable. General demographic information, e.g., gender and age, body mass index (BMI), body temperature, parasite counts, and hematological parameters were reported by number, percentage, geometric mean, and standard deviation. The geometric mean was calculated using the natural logarithm. For analytic statistics, student t test was performed to determine correlations between the means of independent and dependent variables. All statistical analyzes were performed using Statistical Package for the Social Sciences (SPSS 23). A two-sided P value of less than 0.05 was considered significant.

**Results**

A total of 103 patients with malaria were involved in this analysis. Almost all (97.1%) were male and their ages ranged from 18 to 69 years. About one-half were below 30 years of age; the average age of patients seeking malaria screening was 29.75 years (SD = 9.82 years). Most patients (83.5%) presented normal BMI while the rest were underweighted (4.9%) and overweighted (11.7%), respectively. Among patients, 84.5% presented fever at the screening (Table 1).

Among the hematological parameters of 103 patients observed, the hematological change that occurred most, 84.5%, was thrombocytopenia (low platelet counts), and lymphopenia (low lymphocyte counts) at 55.3%. Basophil for nearly one-half (46.6%) of the patients was in a high range. Other parameters were mostly in the normal range (Table 2). *P. falciparum* was diagnosed among all the patients using a blood film examination. The average parasite counts (geometric mean) at the screening was 15 177.97 parasites, with a range from 11 520.69 to 19 996.25 parasites (Table 3).

The parasite counts of the patients were compared along with other independent variables. The average weight seemed higher than other groups (P = 0.047). Patients presenting fever had more parasites than patients without fever (P = 0.003). In hematological parameters, no different parasites were observed among the various level of white blood cells (WBC) and red blood cells (RBCs). However, those with a high level in hematological parameters seemed to have a higher number of parasites than any other group. Regarding hemoglobin and hematocrit, no difference was observed for parasite numbers, but the normal level seemed to be slightly higher when compared with other levels (Table 4).

Mean corpuscular volume (MCV), red cell distribution width (RDW), platelet count, and low levels seemed to be higher than other levels, even though no significant difference was found for parasites for MCV and RDW, while the platelet count was statistically significant (P = 0.039). For monocyte and basophil, no difference was observed in parasite count.

### Table 1. Gender, Age, BMI, and Body Temperature of 103 Patients Participating in Malaria Screening

| Description          | Number | Percent |
|----------------------|--------|---------|
| Gender               |        |         |
| Female               | 3      | 2.9     |
| Male                 | 100    | 97.1    |
| Age (y)              |        |         |
| 18 -19               | 12     | 11.7    |
| 20 -29               | 44     | 42.7    |
| 30-39                | 31     | 30.1    |
| 40-49                | 12     | 11.7    |
| 50-59                | 3      | 2.9     |
| 60-69                | 1      | 1.0     |
| Mean ± SD (y) =29.75 ± 9.82|        |         |

BMI

| Description          | Number | Percent |
|----------------------|--------|---------|
| Underweight          | 5      | 4.9     |
| Normal               | 86     | 83.5    |
| Overweight           | 12     | 11.7    |
| Temperature on screening |      |         |
| Normal               | 16     | 15.5    |
| Fever                | 87     | 84.5    |

### Table 2. Distribution of the 103 Patients by Levels of Hematological Parameters

| Parameters | Low       | Normal | High     |
|------------|-----------|--------|----------|
| WBC        | Number    | Percent| Number   | Percent| Number | Percent|
| RBC        | 12        | 11.7   | 88       | 85.4   | 3      | 2.9    |
| Hemoglobin | 26        | 25.2   | 67       | 65.0   | 10     | 9.7    |
| Hematocrit | 34        | 31.0   | 69       | 67.0   | -      | -      |
| MCV        | 24        | 23.3   | 77       | 74.8   | 2      | 1.9    |
| RDW        | 29        | 26.2   | 62       | 60.2   | 12     | 11.7   |
| Platelet   | 45        | 44.6   | 53       | 52.5   | 3      | 3.0    |
| Neutrophil | 87        | 84.5   | 15       | 14.6   | 1      | 1.0    |
| Lymphocyte | 100       | 97.1   | 94.2     | 1      | 1.0    |
| Monocyte   | 40        | 39.8   | 63       | 61.2   | 20     | 19.4   |
| Eosinophil | -         | -      | 94       | 91.3   | 9      | 8.7    |
| Basophil   | 1         | 1.2    | 53       | 51.5   | 48     | 46.6   |
| Abs-neutrophil | 18   | 17.5   | 83       | 80.6   | 10     | 9.7    |
| Abs-lymphocyte | 17   | 16.6   | 45       | 43.7   | 1      | 1.0    |
| Abs-monocyte | 25     | 24.3   | 67       | 65.0   | 11     | 10.7   |
| Abs-eosinophil | -    | -      | 91       | 88.3   | 12     | 11.7   |
| Abs-basophil | -      | -      | 64       | 62.1   | 39     | 37.9   |

**Table 3. Mean of Parasite Counts Among 103 Patients Participating in Malaria Screening**

| Values     | In(parasite)\(^a\) | Geometric Mean |
|------------|---------------------|----------------|
| Mean       | 9.63                | 15177.97       |
| Standard error mean | 0.14              |                |
| 95% CI for mean |                |                |
| Lower bound | 9.35                | 11520.69       |
| Upper bound | 9.90                | 19996.25       |

*In(parasite): Natural logarithm of parasite.*

Abbreviations: SD, Standard deviation; BMI, body mass index.
among the three levels, but it seemed to be higher at a low level than other reports. Absolute neutrophil and absolute basophil showed no difference in parasite density among those three levels, but low levels seemed to be associated with higher parasite count than other various levels. Among three levels of absolute monocyte, the low level seemed to be higher than other levels, even though no significant difference was observed among them. Parasite counts significantly differed among the three levels of neutrophil ($P < 0.005$), eosinophil ($P < 0.001$), and absolute Eosinophil ($P < 0.001$), whereas the normal level had a higher parasite count than other levels. The difference among three levels of lymphocyte ($P = 0.001$) and absolute lymphocyte ($P = 0.001$) was significant; those at a low level seemed to have higher numbers of parasites than other levels (Table 4).

**Table 4.** The Associations Between Parasite Counts and BMI, Body Temperature, and Hematological Parameters (n = 103)

| Variables             | n    | Mean | SEM   | 95% CI for Mean | P Value | Mean (GM) | 95% CI for Mean (GM) |
|-----------------------|------|------|-------|-----------------|---------|-----------|---------------------|
| **BMI**               |      |      |       |                 |         |           |                     |
| Underweight           | 5    | 9.59 | 0.65  | 7.77-11.40      | 0.047*  | 14,569.71 | 2379.87             |
| Normal                | 86   | 9.76 | 0.14  | 9.47-10.05      | 0.039*  | 17,317.03 | 13,019.45           |
| Overweight            | 12   | 8.69 | 0.48  | 7.63-9.75       | 0.003*  | 5794.13   | 2739.96             |
| **Body temperature**  |      |      |       |                 |         |           |                     |
| Normal                | 16   | 8.66 | 0.35  | 7.92-9.41       | 0.001*  | 18,118.70 | 13,601.02           |
| Fever                 | 87   | 9.80 | 0.14  | 9.52-10.09      | 0.039*  | 7499.56   | 3773.66             |
| **Platelets**         |      |      |       |                 |         |           |                     |
| Low                   | 87   | 9.76 | 0.15  | 9.45-10.06      | 0.001*  | 17,247.11 | 12,756.55           |
| Normal                | 15   | 8.92 | 0.32  | 8.24-9.61       | 0.005*  | 7499.56   | 3773.66             |
| High                  | 1    | 9.08 | -     | -               |         | 8804.34   | -                   |
| **Neutrophil**        |      |      |       |                 |         |           |                     |
| Low                   | 5    | 7.70 | 0.45  | 6.46-8.94       | 0.001*  | 2199.97   | 636.13              |
| Normal                | 97   | 9.74 | 0.14  | 9.46-10.01      | 0.001*  | 16,919.13 | 12,855.15           |
| High                  | 1    | 8.75 | -     | -               |         | -         | -                   |
| **Lymphocyte**        |      |      |       |                 |         |           |                     |
| Low                   | 30   | 10.39| 0.22  | 9.94-10.85      | 0.001*  | 32,646.73 | 20,731.30           |
| Normal                | 72   | 9.33 | 0.16  | 9.01-9.65       | 0.039*  | 11,306.13 | 8195.99             |
| High                  | 1    | 7.85 | -     | -               |         | -         | -                   |
| **Eosinophil**        |      |      |       |                 |         |           |                     |
| Low                   | -    | -    | -     | -               |         | -         | -                   |
| Normal                | 94   | 9.77 | 0.14  | 9.50-10.05      | <0.001* | 17,576.18 | 13,298.41           |
| High                  | 9    | 8.09 | 0.32  | 7.36-8.83       | 0.012*  | 3277.05   | 1572.31             |
| **Abs-Lymphocyte**    |      |      |       |                 |         |           |                     |
| Low                   | 57   | 10.03| 0.17  | 9.68-10.38      | 0.001*  | 22,672.32 | 16,037.74           |
| Normal                | 45   | 9.19 | 0.20  | 8.78-9.59       | 0.001*  | 9786.90   | 6527.64             |
| High                  | 1    | 6.50 | -     | -               |         | -         | -                   |
| **Abs-Eosinophil**    |      |      |       |                 |         |           |                     |
| Low                   | -    | -    | -     | -               |         | -         | -                   |
| Normal                | 91   | 9.82 | 0.14  | 9.54-10.10      | <0.001* | 18,372.31 | 13,920.25           |
| High                  | 12   | 8.18 | 0.33  | 7.44-8.91       | 0.047*  | 3566.36   | 1039.60             |

*Significance at P < 0.05 by t test.

**Discussion**

Gender demographics among participants in this study were almost 100% male and resulted in no significant correlation with parasite counts. Due to the local culture, women remained working around the residence and men worked in the forest, making the men more prone to malaria infections than the women. The analysis also showed that age was unassociated with differences in parasite counts. No significant differences were found among the age groups concerning parasite density, which was similar to the results of the study conducted in Ghana. BMI was significantly associated with parasite count, and patients with normal BMI seemed to have higher parasite counts than overweight and overweight patients. One study conducted in northeastern Myanmar showed similar results, indicating a significant
linkage between parasite counts and patients’ BMI.\textsuperscript{17} Along with the gradual improvement of average gross domestic product, malnutrition among adults becomes rare and increased in proportion to normal BMI.\textsuperscript{18}

Patients with fever had more parasites than patients without fever to a significant degree. Some patients came seeking treatment having just encountered the infection, so had not yet experienced a fever. The results were supported by other relevant studies that proved increasing parasite loads when a patient has malaria fever.\textsuperscript{19,20} Overall, although the results suggest significant outcomes between BMI and body temperature with parasite counts, the majority possessed normal BMI. Increased parasite density during fever could not be considered as a sign or symptom to diagnose malaria clinically.

Among the 103 patients with malaria, the hematological parameters showed only 3% had leukocytosis as the inclusion criteria of this study were only uncomplicated malaria. This finding contradicted other results where malaria exhibited a strong association with leukocytosis as stated in other studies.\textsuperscript{21,22} This study showed the majority of patients, having normal RBC; hemoglobin, hematocrit, MCV, and RDW seemed to be higher than other levels, but around one-third of the participants still presented anemia. Anemia is one of the most common complications of patients with malaria, especially those with infection due to \textit{P. falciparum}.\textsuperscript{22}

In this study, the lower levels of RBC, hemoglobin, hematocrit, MCV, RDW were not significant but all patients with malaria should follow up their hematological parameters to prevent the clinical signs of anemia, which can make the patients’ situation more severe and complicate treatment. In contrast, the study of Maina et al.\textsuperscript{23} showed significant results with low levels of hemoglobin, platelets, and RBC. The absolute type of WBC (neutrophil, lymphocyte, monocyte, eosinophil, and basophil) count and the type of WBC did not significantly differ. The eosinophil and absolute eosinophil counts were strongly correlated with parasite count. This compared with the study of Akhtar S.\textsuperscript{24} showing normal eosinophil count among higher parasite counts than the eosinophilia and eosinopenia groups. In summary, the results described significant associations among neutrophils, lymphocytes, eosinophils, absolute eosinophils, and parasite counts of patients with malaria; most clients showed normal parameters. Hence, it could not probably be used when focusing on the disease.

Meanwhile, the low platelet level or presence of thrombocytopenia was observed to be the highest group in this study and was statistically significant with parasite counts. Thrombocytopenia is the most common indicator of \textit{P. falciparum} malaria even among uncomplicated patients, resulting from destruction in the spleen or the lifespan of platelets becoming shorter.\textsuperscript{25} The exact mechanisms causing thrombocytopenia remain unclearly identified, and could easily be reversible. The condition could return to its normal range even after a course of treatment or within a week after parasites had cleared.\textsuperscript{18,26} Other similar results can be found in Khan and colleagues’ and Niazi’s papers, in which 53% and 57% of malaria cases presented with thrombocytopenia, respectively.\textsuperscript{27,28} Thus, platelet count can be used to predict malaria as a clinical marker with, as shown by some studies, a greater than 80% specificity in an adult population.\textsuperscript{27,29,30}

In this study, one-half of the participants presented absolute lymphocytopenia showing a significant association with parasite counts. This lymphocytopenia might be the outcome of designating lymphocytes towards sites of inflammation.\textsuperscript{18} Nevertheless, levels ranged back to normal within one week of taking anti-malarial drugs.\textsuperscript{18,20,25} Higher parasite density seems to produce more marked lymphocytopenia, but one study found that only 24% was noticed in an adult rural community.\textsuperscript{30}

**Conclusion**

The study provided information about the effects of \textit{P. falciparum} parasites on hematological parameters and classified their underlying risk factors. The results concluded that BMI could influence parasite density whereas parameters, mainly thrombocytopenia, and lymphocytopenia, were the consequences of infection. It appears likely that thrombocytopenia and lymphocytopenia could act as appropriate clinical markers in diagnosing uncomplicated acute \textit{P. falciparum} infection. Hence, we recommended properly investigating hematological assays among every confirmed \textit{P. falciparum} malaria case. Renal functions such as urea and creatinine should also be checked to confirm the severity of malaria.
Authors’ Contributions
RB and PLA conceptualized the study. RB supervised the project. All authors involved in data curation, formal analysis, writing and revising the original draft, and approved the final manuscript.

Conflict of Interest Disclosures
The authors declared no conflict of interest.

Ethical Approval
The ethics approval was sought by the Ethics Committee for Human Research under the Faculty of Public Health, Mahidol University with documentary proof of exemption (Ref. No. 73/2558).

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