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Hematological Abnormalities And Their Determinants Among Patients Infected With Plasmodium Falciparum And Plasmodium Vivax In Bahir Dar, Northwest Ethiopia: A Cross-Sectional Study

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BAHIR DAR UNIVERSITY
COLLEGE OF MEDICINE AND HEALTH SCIENCES
SCHOOL OF MEDICAL LABORATORY SCIENCE
DEPARTMENT OF CLINICAL HEMATOLOGY AND
IMMUNOHEMATOLOGY

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Vivax In Bahir Dar, Northwest Ethiopia: A Cross-Sectional Study**

By:

Mr. Abraham Dessie

(MSc candidate in Clinical Hematology and Immunohematology)

August 2026

Bahir Dar, Ethiopia



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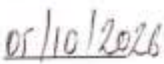
A Thesis Submitted to the Department of Clinical Hematology and Immunohematology, School of Medical Laboratory Science, College of Medicine and Health Sciences, Bahir Dar University, in Partial Fulfillment of the Requirements for the Degree of Master of Science in Clinical Hematology and Immunohematology.



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List of acronyms and abbreviations

AOR	Adjusted Odds Ratio
CBC	Complete Blood Count
CI	Confidence Interval
COR	Crude Odds Ratio
EDTA	Ethylenediaminetetraacetic Acid
HCT	Hematocrit
HGB	Hemoglobin
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MPV	Mean Platelet Volume
<i>P. falciparum</i>	<i>Plasmodium falciparum</i>
<i>P. vivax</i>	<i>Plasmodium vivax</i>
PCR	Polymerase Chain Reaction
PCT	Plateletcrit
PDW	Platelet Distribution Width
PI	Principal investigator
PLT	Platelet Count
PPE	Personal Protective Equipment
QA	Quality Assurance
QC	Quality Control
RBC	Red Blood Cell
RDW	Red Cell Distribution Width
RDW-CV	Red Cell Distribution Width Coefficient of Variation
SOP	Standard Operating Procedure
SPSS	Statistical Package for Social Science
UAE	United Arab Emirates
WBC	White Blood Cell
WHO	World Health Organization

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Abstract

Background: Malaria is an infectious parasitic disease caused by protozoa of the genus *Plasmodium*. Among these, *Plasmodium falciparum* and *Plasmodium vivax* are the most common species infecting humans. These species cause various hematological abnormalities, including anemia, thrombocytopenia, and leukopenia. However, evidence on the prevalence and determinants of these abnormalities between these species remains limited and inconsistent among malaria patients in Bahir Dar city.

Objectives: To assess hematological abnormalities and their determinants among patients infected with *Plasmodium falciparum* and *Plasmodium vivax* in Bahir Dar City.

Methods and Materials: An institution-based comparative cross-sectional study was conducted from February 3 to April 10, 2026. Venous blood samples were collected from participants confirmed to have *Plasmodium falciparum* or *Plasmodium vivax* infection for complete blood count (CBC) analysis. Data were cleaned, coded, and entered into statistical package for social science version 25. The Chi-square test was used to assess associations between categorical variables. An independent samples t-test was used to compare means of continuous variables. Binary logistic regression was used to identify determinants of hematological abnormalities. A p-value < 0.05 was considered statistically significant.

Results: Among 462 participants, 231 were infected with *Plasmodium falciparum* and 231 with *Plasmodium vivax*. The majority of participants were male, 253 (54.8%). The most encountered hematological abnormality was thrombocytopenia (57.36%), followed by anemia (31.39%). Thrombocytopenia and anemia were significantly higher among participants infected with *Plasmodium falciparum* than those with *Plasmodium vivax*, with AORs of 1.895 (CI: 1.243–2.887) and 3.476 (CI: 1.838–6.574), respectively. Previous malaria infection was significantly associated with thrombocytopenia (AOR = 1.788, CI: 1.037–3.082).

Conclusion: Hematological abnormalities were common among malaria-infected patients, with higher occurrence in *Plasmodium falciparum* than *Plasmodium vivax*. Several determinants were associated with these abnormalities. Therefore, routine hematological assessment, early species-specific diagnosis, and swift treatment are recommended to improve patient outcomes.

Keywords: Hematological abnormalities; Determinants; Malaria; *Plasmodium falciparum*; *Plasmodium vivax*.

1. Introduction

1.1. Background

Malaria is a vector-borne infectious parasitic disease caused by different species of *Plasmodium*. It is mostly transmitted to humans through the bite of an infected female *Anopheles* mosquito during blood feeding(1). More than 200 *Plasmodium* species have been identified, but only five species are known to infect humans, such as *Plasmodium falciparum* (*P. falciparum*), *Plasmodium vivax* (*P. vivax*), *Plasmodium malariae* (*P. malariae*), *Plasmodium ovale* (*P. ovale*), and *Plasmodium knowlesi* (*P. knowlesi*)(2).

Malaria, mainly caused by *P. falciparum* and *P. vivax*, remains a major global health problem. These species are responsible for the majority of malaria-related deaths worldwide (3). According to the World Health Organization (WHO) report, globally about 249 million malaria cases were documented in 2022, resulting in an estimated 608,000 deaths. Around 95% of these cases and deaths occurred in sub-Saharan Africa, where *P. falciparum* is responsible for the majority of the fatalities(4). In Ethiopia, the Federal Ministry of Health (FMOH) reported that about three-quarters of its territory is considered endemic for malaria, placing more than 60 million (60% of the total population) people at risk for infection (5).

The life cycle of malaria is complex and involves both humans and female *Anopheles* mosquitoes. In humans, an infected female *Anopheles* mosquito inoculates sporozoites. These sporozoites migrate to the liver, where they multiply and release merozoites into the bloodstream. These merozoites invade red blood cells (RBCs), where they develop through ring and trophozoite stages, leading to repeated RBC destruction, hemolysis, and anemia. Some parasites differentiate into gametocytes, which are subsequently taken up by mosquitoes during blood feeding (6).

The pathophysiology of malaria involves a complex cascade of events driven by the metabolic by-products of the parasite, hemoglobin breakdown products, dysregulated inflammatory cytokine responses, and the cytoadherence of infected RBCs to the vascular endothelium. These processes result in sequestration and rosette formation, contributing to numerous hematological abnormalities (7).

Changes in blood cell counts are a well-known feature of malaria infection, affecting all major blood cell lines, including RBCs, white blood cells (WBCs), and platelets. Key hematological parameters such as RBC count, total WBC count, platelet count, hemoglobin (HGB) concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW) are commonly evaluated to assess the impact of malaria on the hematological system (8).

Thrombocytopenia, anemia, and leukopenia are among the most common hematological abnormalities associated with malaria. The prevalence and severity of these abnormalities differ among *Plasmodium* species (9). Malaria-induced anemia is a serious complication predominantly caused by destruction of parasitized RBCs (10). Thrombocytopenia due to malaria is mainly attributed to immune-mediated platelet destruction and splenic sequestration (11).

Regarding malaria-induced WBC abnormalities, lymphocytes and neutrophils are the most commonly affected WBCs, resulting in leukocytopenia, lymphocytopenia, and neutropenia. These changes are primarily driven by immune-mediated redistribution, splenic sequestration, and systemic inflammatory responses during infection (12). The extent of these hematological abnormalities varies with malaria species, as well as clinical and socio-demographic factors (13). Therefore, this study aims to assess hematological abnormalities and their determinants among *P. falciparum* and *P. vivax*-infected patients in Bahir Dar City.

1.2. Statement of the problem

Malaria remains one of the biggest health problems in the world despite substantial improvement in control and prevention efforts. According to the WHO 2022 report, malaria cases increased globally in 2020 and 2021, with 245 million and 247 million cases reported, respectively (14). The 2023 WHO report further indicated approximately 608,000 malaria-related deaths occurred worldwide in 2022, with African countries accounting for more than half of these deaths (15).

Among various *Plasmodium* species, *P. falciparum* and *P. vivax* account for the majority of cases and deaths. Globally, in 2022, *P. falciparum* accounted for 244.4 million cases, whereas *P. vivax* was responsible for about 12.43 million cases (3). In Ethiopia, malaria remains a significant public health concern, with the Amhara Region representing a persistent hotspot due to its diverse geography. This region has contributed about 18% of all confirmed cases in the country in 2024. Over the 11-year period from 2014 to 2024, the Amhara Region reported a total of 7,710,733 malaria cases and 162 deaths (16).

Beyond its health impact, malaria has also become a major economic burden for many countries throughout the world. Between 2000 and 2016, total malaria-related spending in 106 countries increased at an annual rate of 8.5%, rising from US\$1.2 billion in 2000 to US\$4.3 billion in 2016 (17). Furthermore, in 2017, the estimated global healthcare provider and household cost in 44 *P. vivax*-endemic countries amounted to US\$359 million (18).

Malaria due to *P. falciparum* and *P. vivax* causes several hematological abnormalities. Anemia, thrombocytopenia, and WBC-related abnormalities are among the common abnormalities that occur due to these species (19). However, existing studies conducted in the world reported that patients infected with *P. falciparum* and *P. vivax* exhibit differences in the prevalence of these hematological abnormalities (8). For instance, a study conducted in Iran showed that *P. falciparum* infection had lower RBC parameters, WBC counts, and platelet counts as compared with *P. vivax* infections (20).

However, these findings cannot be generalized across different parts of the world due to variations in host immunity and local epidemiology. Moreover, most previous studies have focused on hematological abnormalities in specific age groups, such as children or adults, which limit the generalizability of their findings. Therefore, this study addresses this gap by providing comprehensive evidence across all age groups, ranging from neonates (0–28 days) to older adults (≥ 60 years).

In Ethiopia, especially in Bahir Dar, evidence comparing hematological abnormalities between *P. falciparum* and *P. vivax* remains scarce. Moreover, hematological parameters can vary over time due to changes in vector, parasite, and host factors, which suggests that the existing evidence may not reflect the current conditions. Consequently, clinicians lack sufficient locally generated, species-specific hematological data to guide their accurate early diagnosis, prognostic evaluation, and effective management for different malaria species.

Furthermore, in the current study area, evidence regarding the association between determinants and hematological abnormalities among malaria-infected patients has not been well studied, which restricts the comprehensive understanding of the contributing factors. Therefore, this study aims to address this gap by providing locally generated data on the differences in hematological abnormalities and their determinants among patients infected with *P. falciparum* and *P. vivax* in Bahir Dar, Northwest Ethiopia.

1.3. Significance of the study

Till now, malaria has been a major public health issue, especially in the areas where it is commonly present. *P. falciparum* and *P. vivax* cause different types of hematological abnormalities through different mechanisms such as increasing RBC destruction and causing anemia, reducing platelet count and thereby increasing the likelihood of bleeding, and reducing leukocyte count, which may lead to an increased risk of secondary infections and increase morbidity and mortality in malaria-infected patients.

Determining the prevalence of these hematological abnormalities among *P. falciparum* and *P. vivax*-infected patients provides local epidemiological data for researchers and physicians, and supports early detection and management of potential complications. Knowing the species-specific effect of hematological abnormalities is also important for predicting clinical outcomes and adjusting treatment and monitoring plans accordingly.

In addition, assessing the severity of anemia and thrombocytopenia among patients infected with *P. falciparum* and *P. vivax*, which helps physicians to identify high-risk individuals, provide closer follow-up to reduce adverse effects on patients' health, predict disease outcomes and allocate resources efficiently. As well, this study supports the use of routine hematological parameter assessment techniques such as complete blood count, which is an inexpensive and widely available tool that provides rapid clinical insights particularly in resource-limited areas, aiding in patient stratification, and early detection of severe cases.

Moreover, it provides important information on determinants associated with different hematological abnormalities among *P. falciparum* and *P. vivax*-infected patients. Understanding these determinants is essential for effective clinical management and prevention of malaria-related hematological problems. In addition, it serves as a vital contribution for future systematic reviews and meta-analyses. Generally, this study provides locally appropriate evidence that strengthens medical decisions, guides public health interventions, helps timely patient care and follow-up strategies, and provides clear information for scholars about hematological abnormalities caused by *P. falciparum* and *P. vivax*.

2. Literature review

2.1. The overall malaria-associated hematological abnormalities

Malaria is associated with several hematological abnormalities, with studies reporting varying prevalence rates across different settings. For instance, comparative cross-sectional studies conducted in Nagpur (India), Dubai (United Arab Emirates (UAE)), Rabat (Morocco), Mtwara (Tanzania), Edo State (Nigeria), and Harar (Ethiopia) reported malaria-induced anemia in 70 (94%) (21), 85 (64%) (22), 321(90%) (23), 129 (61%) (24), 122 (38.6%) (25), and 120 (47%) (26) patients, respectively. Similarly, malaria-induced thrombocytopenia was reported in 52 (70%) (21), 55 (58%) (27), 110 (83%) (22), 300 (84%) (23), and 172 (67.7%) (26) patients in Nagpur (India), Peshawar (Pakistan), Dubai (UAE),Rabat (Morocco), and Harar (Ethiopia), respectively.

White blood cell-related abnormalities have also been reported among malaria-infected patients in different countries. For instance, lymphopenia was reported in Nagpur (India), Dubai (UAE), and Rabat (Morocco) among 9 (12%) (21), 32 (24%) (22), and 178 (78%) (23)patients, respectively. In addition, leukopenia was observed in 160 (18%) of patients in Medellin(Colombia)(28) and 81 (31.9%) in Harar (Ethiopia)(26). The study conductedin Harar, Ethiopiafurther reported leukocytosis in 33 (13%) of participants (26). Furthermore, neutropenia (8%) and neutrophilia (4%) were also documented in Rotterdam (Netherlands)(29).

2.2. Hematological abnormalities in *Plasmodium falciparum* infection

2.2.1. Anemia

Numerouscomparative cross-sectional studies in malaria-endemiccountries reported that the incidence of anemia among *P. falciparum*-infected patients varies widely, ranging from 34% to 67%. For instance, the prevalence rates of 46.7% in Merauku, Indonesia (30), 55.3% in Rio de Janeiro, Brazil (31), 55.5% in Volta, Ghana (32), 67% in Accra, Ghana(33), 63.64% in Bamenda Regional Hospital, Cameroon (34), 60.4% in Kosti city, Sudan (35), and 65.7% in Dawuro, Ethiopia(36) have been documented, despite the fact that a lower prevalence of 34% was reported at Raya Alamata Hospital, Ethiopia (37).

2.2.2. Thrombocytopenia

The prevalence of thrombocytopenia in *P. falciparum* infection also varied across countries, ranging from 14.9% to 96%. For instance, it was 72% in Pune, India (38), 26% in Khyber Pakhtunkhwa, Pakistan (39), 96% in Makkah, Saudi Arabia (40), 47.46% in Volta Regional Hospital, Ghana (32), 14.9% in slope of Mount Cameroon (41), 49% in KisumuWest District Kenya (42), 72.4% in Kosti, Sudan (35). In Ethiopia, the prevalence was 46.3% in Raya Alamata hospitals(37) and 83% in Dawuro (36).

2.2.3. Leukocyte-related abnormalities

Leukopenia frequency among *P. falciparum*-infected patients shows broad variation, ranging from 12.5% to 94.9%. For example, 42% was reported in Khammam, India (43), 94.92% in Bamenda Regional Hospital,Cameroon (34),12.5% in Kosti city, Sudan (35), 52.4% in Dawuro, Ethiopia (36), and 74.1% in Raya Alamata Hospital, Ethiopia (37). In addition, leukocytosis was reported in 36 (17.4%) patients in Volta Region, Ghana (32) and 3 (2.52%) in Raya Alamata Hospital, Ethiopia (37).

In Volta Regional Hospital Ghana, the prevalence rates of neutrophilia, lymphopenia, and neutropenia was 25 (10.59%), 117 (56.78%) and 60 (29.24%), respectively(32). Similarly, in Kosti city, Sudan, 51 (26.6%) patients developed neutropenia and 11 (5.7%) developed lymphopenia(35). Moreover, at Tercha General Hospital, Dawuro, Ethiopia, lymphopenia and neutrophilia were observed in 59 (56%) and 22 (21%) of patients, respectively (36).

2.3. Hematological abnormalities in *Plasmodium vivax* infection

2.3.1. Anemia

Anemia occurrence among *P. vivax*-infected patients varied widely across countries. In Merauke, Indonesia, 21% of patients developed anemia(30). In Rio de Janeiro, Brazil, 55.3% were anemic (31), while in Merida State, Venezuela, prevalence ranged from 94.9% to 96% (44, 45). In Ethiopia, prevalence ranged from 29.9% to 52.4%, with Tercha General Hospital reporting 52.4% (36), Raya Alamata Hospital (48.9%)(37), and Mendi town (29.9%), among whom 23.6% were severely anemic (46).

2.3.2. Thrombocytopenia

In addition, a number of comparative cross-sectional studies have reported that the incidence of thrombocytopenia among *P. vivax*-infected participants varies widely across different regions, including 10.9% in Medellin, Colombia (47), 55.4% in Khyber Pakhtunkhwa, Pakistan (39), 58.97% in Merida State, Venezuela (44), and 86.8% in Makkah, Saudi Arabia (40). In Pune, India, 58% of patients developed thrombocytopenia (38) and in Ethiopia, prevalence ranged from 40.3% to 85% , including 85% in Dawuro (36), 40.3% Raya Alamata (37), and 43% Kersa/Halaba Kulito Hospitals (48).

2.3.3. Leukocyte-related abnormalities

Several comparative cross-sectional studies from different countries reported wide variation in leukocyte abnormalities among *P. vivax* infections. Leukopenia ranged from 4.5% in Merida State, Venezuela (49) to 45.9% in Karang Puskesmas, Indonesia (50). Intermediate prevalence was reported in Khammam and Firozabar, India (10–30%) (43, 51), Khyber Pakhtunkhwa, Pakistan (21.2%) (52), Dubai, UAE (14%) (22), Dawuro, Ethiopia (44%) (36), and Raya Alamata, Ethiopia (15.5%) (37). In addition, leukocytosis was observed in 10% of patients in Firozabad, India (51), 78.8% in Khyber Pakhtunkhwa, Pakistan (52), 2% in Dubai, UAE (22), and 3.4% in Raya Alamata, Ethiopia (37).

Additional studies have reported other leukocyte abnormalities associated with *P. vivax* infection. For instance, in Karang Puskesmas, Indonesia, 1 (2.70%) patient developed neutrophilia, 1 (2.70%) lymphocytosis, 4 (10.80%) neutropenia, and 13 (35.10%) lymphopenia (50). In Merida State, Venezuela, 18 (13.5%) of patients were lymphopenic, and 22 (16.5%) were neutropenic (49). Correspondingly, in Dubai, UAE, 26 (36%) participants developed lymphopenia (22). Moreover, in Dawuro, Ethiopia, 33 (54%) developed lymphopenia and 23 (37.7%) neutrophilia (36).

2.4. Determinants of malaria-induced hematological abnormalities

2.4.1. Socio-demographic factors

Sex differences in hematological abnormalities are inconsistent. Comparative cross-sectional studies in different countries have reported females were more thrombocytopenic than males; however, no significant association between sex and thrombocytopenia was observed in Jamshoro, Pakistan ($p = 0.320$)(53) and Kigeme District Hospital, Rwanda ($p = 0.689$) (54). Regarding anemia, a Ugandan malaria indicator survey showed that females were significantly associated with lower odds of anemia (AOR = 0.797, CI: 0.713–0.891)(55), but in Harar, Ethiopia female sex was significantly associated with higher odds of anemia (AOR = 3.18, CI: 1.671–6.038)(26).

A comparative cross-sectional study from Kigeme District Hospital, Rwanda reported that children aged ≤ 5 years had significantly increased odds of severe thrombocytopenia compared with older patients (AOR = 5.17, 95% CI: 1.674–16.259, $p = 0.004$) (54), but in Harar, Ethiopia, there was no significant association (26). Regarding anemia, in Ibadan, Nigeria, children under 5 years of age had a significantly lower hematocrit compared with those older than 5 years ($28.4 \pm 4.8\%$ vs $32.8 \pm 4.8\%$; $p < 0.0001$) (56).

Similarly in Shoklo camp, Thailand, children under 5 years were 2.6 times more likely than 5–14-year-olds and 3.6 times more likely than adults to be anemic (57), but in Harar, Ethiopia, there was no significant association of age and anemia (26). Concerning other socio-demographic factors, in Harar, Ethiopia, reported no significant association between hematological abnormalities (thrombocytopenia and anemia) and residence, occupation, and educational status (26).

2.4.2. Clinical factors

A retrospective study in Shoklo camp, Thailand showed that patients who had prolonged duration of illness before treatment had significantly higher prevalence of anemia compared with those who had short duration (AOR, 1.5, 95% CI, 1.3–1.7; $p < 0.001$)(57). Similarly, in Phop Phra Hospital, Thailand these recurrent cases showed significant alterations in hematological parameters, specifically leukocyte counts and RDW compared with non-recurrent cases (58). In addition, in Ibnu Sina Hospital, Indonesia patients with a history of previous malaria infection had significantly lower hemoglobin levels and platelet counts compared with those without prior infection, but no significant association was observed between malaria history and leukocyte counts (59).

Generally, this literature indicates that hematological abnormalities are the common consequences caused by *P. falciparum* and *P. vivax* infections. However, the prevalence and the types of these hematological abnormalities vary by their geographical location, socio-demographic as well as clinical factors, so this variation highlights the need for local studies to fully understand the influences of malaria on hematological profiles.

3. Conceptual framework

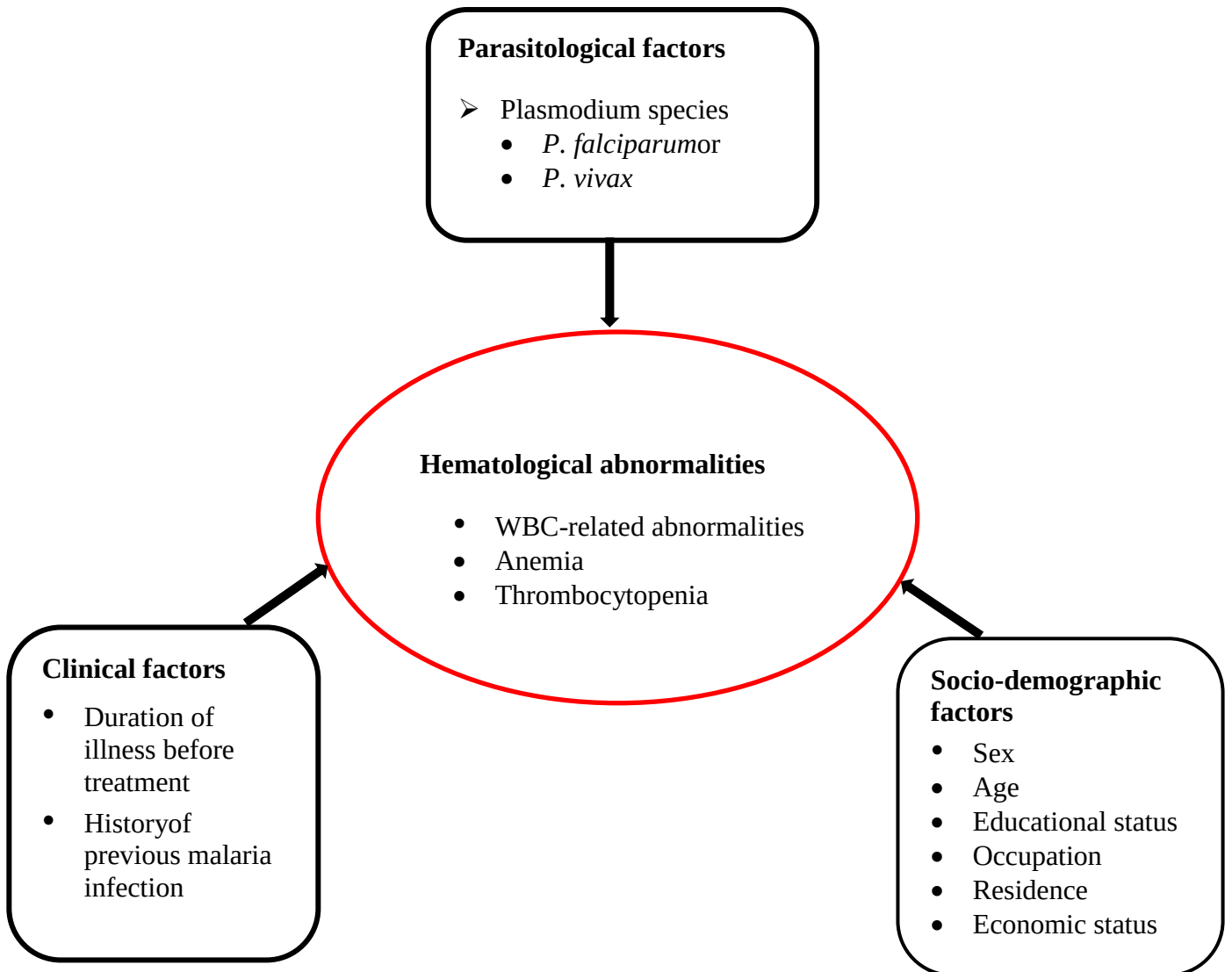


Figure 1: Conceptual framework illustrating determinants of hematological abnormalities among patients infected with *P. falciparum* and *P. vivax*.

4. Objectives

4.1. General objective

To assess hematological abnormalities and their determinants among patients infected with *P. falciparum* and *P. vivax* attending selected public health institutions in Bahir Dar, Ethiopia.

4.2. Specific objectives

- To assess the prevalence of hematological abnormalities among malaria-infected patients.
- To compare the hematological abnormalities among *P. falciparum* and *P. vivax*-infected patients.
- To identify determinants of hematological abnormalities among malaria-infected patients.

5. Methods and Materials

5.1. Study area

The study was conducted at selected public health institutions in Bahir Dar City, the capital of the Amhara Regional State. The city is located between 11°30'–11°41' N latitude and 37°16'–37°30'E longitude, with an altitude ranging from 1717 to 2008 m above sea level. It covers a total area of approximately 213.55 km² and is situated in the northwest part of Ethiopia, about 565 km from Addis Ababa, the capital city of Ethiopia(60). According to the 2022 Bahir Dar City Administration report, the total population of the city was approximately 345,088 (61).

The city has ten public health centers (Abay, Bahir Dar, Dagmawi Minilik, Han, Meshenti, Shimbit, Shumabo, Tis Abay, Zege, and Zenzelma)(62) and three governmental hospitals(two comprehensive specialized hospitals and one primary hospital)(63). For this study, four public health institutions (Han Health Center, Shimbit Health Center, Shumabo Health Center, and Addis Alem Primary Hospital) were selected using a lottery method.

5.2. Study design and period

An institution-based comparative cross-sectional study was conducted from February 3 to April 10, 2026.

5.3. Population

5.3.1. Source population

All patients clinically suspected of malaria who visited the selected health institutions during the study period were considered the source population.

5.3.2. Study population

Patients clinically suspected of malaria who attended the medical laboratory sections of selected public health institutions in Bahir Dar City during the study period and fulfilled the inclusion criteria.

5.4. Inclusion and exclusion criteria

5.4.1. Inclusion criteria

Patients whose peripheral blood smear results were positive for *P. falciparum* or *P. vivax* and who gave their consent (guardian consent for minors) to participate in this study were included.

5.4.2. Exclusion criteria

Patients with a medical history of chronic infections (HIV/AIDS, tuberculosis, renal disease, and liver disease); patients with a history of known pre-existing hematological disorders (sickle cell disease, leukemia, and thalassemia); pregnant women; patients who received blood during the last 3 months; patients taking medications known to affect hematological parameters (chemotherapy, heparin, and immunosuppressants); patients who had received antimalarial treatment within the past 1 month; patients with laboratory-confirmed intestinal parasitic infections (*Ascaris lumbricoides*, *Giardia lamblia*, *Entamoeba histolytica*, *Trichuris trichiura*, and Hookworm species); and patients with mixed malaria infection were excluded from this study.

5.5. Variables

5.5.1. Dependent variables

In this study, anemia, thrombocytopenia, leukopenia, leukocytosis, neutropenia, neutrophilia, lymphopenia, and lymphocytosis were considered as dependent variables.

5.5.2. Independent variables

In this study, malaria species (*P. falciparum* and *P. vivax* infections), age, sex, residence, educational status, occupation, economic status, duration of illness before treatment, and history of previous malaria infection were considered as independent variables.

5.6. Sample size and sampling technique

5.6.1. Sample size

The sample size was calculated using two-population proportion formula, taking a previous study conducted at Tercha General Hospital, Dawuro, Ethiopia, as a reference. Among the hematological abnormalities, anemia was used for sample size determination due to its largest difference between the groups (13.3%)(36).

The sample size was calculated using the following formula:

$$n = \left(\frac{Z_{\alpha}}{2} + Z_{\beta} \right)^2 \times \frac{p_1(1 - p_1) + p_2(1 - p_2)}{(p_1 - p_2)^2}$$

where n is the number of study participants in each group, P1 is the proportion of anemia in *P.falciparum* (0.657), P2 is the proportion of anemia in *P.vivax* (0.524), $Z_{\alpha/2}$ is the value for 95% confidence interval (1.96), and Z_{β} represents 80% power (0.84). Using the above formula the calculated total sample size was 420 participants, with 210 participants allocated to each group. An equal allocation ratio ($n_1 : n_2 = 1:1$) was used to ensure comparable representation of *P. falciparum*- and *P. vivax*-infected participants. After adding 10% allowance for possible non-responses, the final sample size became 462 participants.

5.6.2. Sampling technique

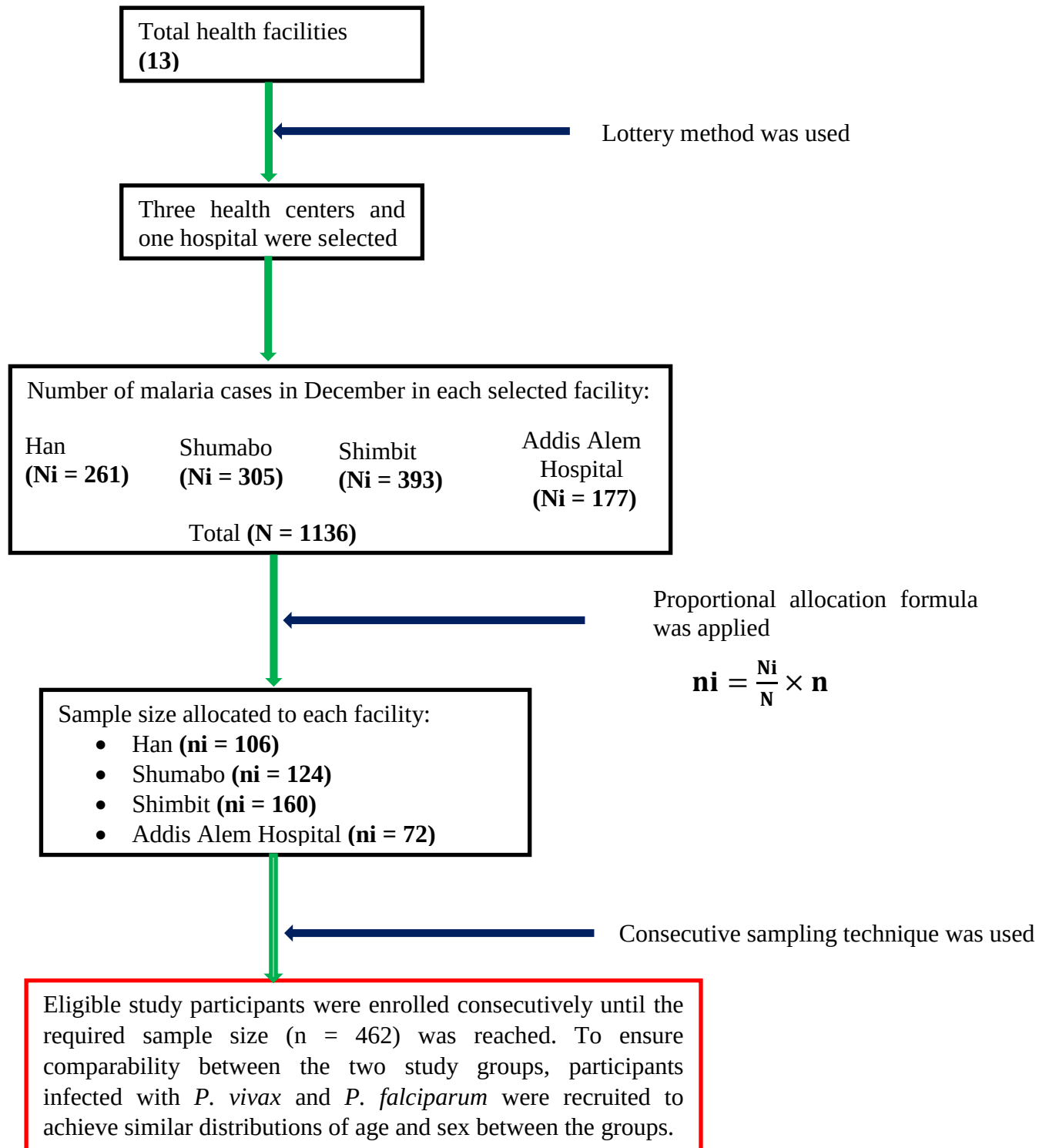


Figure 2: Sampling technique and study participants' recruitment procedure.

5.7. Data collection

5.7.1. Socio-demographic and clinical data collection

The socio-demographic and clinical data were collected from participants using a structured questionnaire translated into the Amharic language, which was pretested at Han Health Center. The questionnaire was administered through face-to-face interviews by the principal investigator (PI) and trained data collectors, while the rights and confidentiality of the study participants were maintained ([Annex 7](#)).

5.7.2. Capillary blood collection and laboratory analysis

Capillary blood was collected from each febrile patient using sterile lancets. A small drop of blood was used to prepare a thin blood smear, while a relatively larger drop was used to prepare a thick blood smear on the same microscopic slide. The thick smear allowed detection of the presence of parasites, whereas the thin smear allowed species identification after being fixed with methanol ([Annex 10](#)). After air-drying and fixation, the slides were stained with Giemsa stain following standard procedures ([Annex 12](#)). Finally, microscopic examination was performed to diagnose malaria and identify the infecting *Plasmodium* species ([Annex 13](#)).

5.7.3. Stool sample collection and laboratory analysis

Participants who tested positive for malaria were instructed to collect approximately a pea-sized amount of fresh stool in a clean, dry, leak-proof, screw-capped container, following standard operating procedures (SOPs). They were also instructed to avoid contamination of the stool sample with urine, water, or soil during collection. The collected samples were properly transported to the laboratory for analysis ([Annex 15](#)).

5.7.4. Venous blood collection and laboratory analysis

Four milliliters (4 mL) of ethylenediaminetetraacetic acid (EDTA)-anticoagulated venous blood were collected aseptically from each study participant ([Annex 11](#)). CBC was performed using semi-automated three-part differential hematology analyzers (Mindray BC-20S, Zybio, and HTI MicroCC-20Plus) to assess hematological parameters, including HGB concentration, HCT, RBC count, MCV, MCH, MCHC, RDW, total WBC count, differential leukocyte count, platelet count, plateletcrit (PCT), mean platelet volume (MPV), and platelet distribution width (PDW) ([Annex 14](#)). Finally, the laboratory-generated results were recorded accordingly.

5.8. Operational definition

- Anemia was defined based on HGB concentration (g/dL). For children aged up to 59 months, non-anemia was defined as ≥ 11.0 g/dL, mild as 10.0–10.9 g/dL, moderate as 7.0–9.9 g/dL, and severe as < 7.0 g/dL. For children aged 5–11 years, non-anemia was defined as ≥ 11.5 g/dL, mild as 11.0–11.4 g/dL, moderate as 8.0–10.9 g/dL, and severe as < 8.0 g/dL. For children aged 12–14 years, non-anemia was defined as ≥ 12.0 g/dL, mild as 11.0–11.9 g/dL, moderate as 8.0–10.9 g/dL, and severe as < 8.0 g/dL. For non-pregnant women aged ≥ 15 years, non-anemia was defined as ≥ 12 g/dL, mild as 11.0–11.9 g/dL, moderate as 8.0–10.9 g/dL, and severe as < 8.0 g/dL. For men aged ≥ 15 years, non-anemia was defined as ≥ 13.0 g/dL, mild as 11.0–12.9 g/dL, moderate as 8.0–10.9 g/dL, and severe as < 8 g/dL (64).
- Thrombocytopenia was defined as a platelet count below $150 \times 10^9/L$ (65), mild as 101–149 $\times 10^9/L$, moderate as 51–100 $\times 10^9/L$, severe as 21–50 $\times 10^9/L$, and very severe as $\leq 20 \times 10^9/L$ (66).
- Leukopenia was defined as leukocyte count $< 4.0 \times 10^9/L$ (65), leukocytosis as $> 10 \times 10^9/L$ (67), lymphopenia as lymphocyte count $< 1.0 \times 10^9/L$, lymphocytosis as $> 4.0 \times 10^9/L$, neutropenia as neutrophil count $< 1.5 \times 10^9/L$, and neutrophilia as $> 7.0 \times 10^9/L$ (29).
- Age categories were defined as follows: neonate (0–28 days), infant (29 days–11 months), child (12 months–9 years), adolescent (10–19 years), young adult (20–24 years), adult (25–44 years), middle-aged adult (45–59 years), and older adult (≥ 60 years) (68, 69).
- Household economic status was defined as low economic status included households with poor-quality housing materials (mud walls, earth floors, and thatched roofs), limited access to safe water, lack of electricity, and inadequate sanitation. Medium economic status was defined households with improved housing materials (brick walls, corrugated iron roofs), access to tap water inside or outside the house and electricity, and basic sanitation facilities. High economic status was defined households with high-quality construction (concrete or stone walls, tiled floors), reliable access to piped water and electricity, improved (high) sanitation, and better household assets and income (70, 71).

5.9. Data quality assurance

The questionnaires were translated into the local language (Amharic), and a pretest was conducted on 5% of the total sample size in Han Health Center to ensure the clarity and consistency of data collection tools. SOPs were followed in all laboratory procedures, and the data collectors were trained in proper data collection. Before conducting the procedures, the quality of Giemsa stains was assessed using known positive and negative blood films with each new batch of working solution. Similarly, the quality of CBC reagents was assessed by checking their expiration dates and storage conditions, in addition to performing quality control (QC) for each batch of reagents. For the automated hematological analyzers, a daily quality control program was performed in accordance with the manufacturer's recommendations.

5.10. Data processing and analysis

All data were cleaned, checked for completeness, and categorical variables were coded numerically before being entered into SPSS version 25 for analysis. Descriptive statistics were used to summarize the socio-demographic and clinical characteristics of study participants. Normally distributed continuous variables were presented as means and standard deviations, whereas non-normally distributed continuous variables were presented as medians.

The association between two categorical variables was analyzed using the Chi-square test. The independent samples t-test was used to compare continuous variables between groups. The association between determinants and hematological abnormalities was analyzed using the binary logistic regression, and variables with a p-value < 0.25 in the bivariable analysis were incorporated in the multivariable logistic regression model. Results were presented using tables and figures. A p-value < 0.05 was considered statistically significant.

5.11. Ethical consideration

Before commencement of the study, ethical approval was obtained from the Institutional Review Board (IRB) of the College of Medicine and Health Sciences, Bahir Dar University. Subsequently, a support letter was written by the College to Amhara Public Health Institute (APHI), which in turn provided additional support letters to each selected health center. Informed consent was obtained from all participants or their guardians after explaining the purpose, procedures, potential risks, and benefits of the study in a language they could understand. Confidentiality was strictly maintained by assigning unique identification codes and restricting data access to authorized personnel only. Study participants who had any laboratory or clinical findings received routine intervention for the respective problem in consultation with clinicians. All biological samples were handled with strict precautions following safety rules and guidelines to protect health personnel and the community.

6. Result

6.1. Socio-demographic and clinical characteristics of study participants

A total of 462 malaria-infected participants were involved. Of these, half (231) were infected with *P. falciparum* and the remaining half were infected with *P. vivax*, with a median age of 20 years. Among the study participants, the majority were males 253(54.8%), urban residents 339 (73.4%), students 191 (41.3%), and those with low economic status 331 (71.6%). The highest number of participants 130 (28.1%) were in the age range of 25–44 year old, while the lowest proportion were observed among neonates (0–28 days) 11 (2.4%). Regarding clinical characteristics, 218 (47.2%) of participants had a duration of illness of ≤ 2 days ([Table 1](#)).

Table 1: Socio-demographic and clinical characteristics of study participants infected with *P. falciparum* and *P. vivax*.

Variables	Categories	Malaria species		Total N (%)
		<i>P. falciparum</i> N(%)	<i>P. vivax</i> N (%)	
Sex	Male	133 (52.6)	120 (47.4)	253(54.8)
	Female	98 (46.9)	111(53.1)	209 (45.2)
	Total	231 (100.0)	231 (100.0)	462(100.0)
Age	0–28 days	5 (45.5)	6 (54.5)	11 (2.4)
	29 days–12 months	14 (53.8)	12 (46.2)	26 (5.6)
	1–9 years	40 (51.9)	37 (48.1)	77 (16.7)
	10–19 years	57 (50.0)	57 (50.0)	114 (24.7)
	20–24 years	32 (48.5)	34 (51.5)	66 (14.3)
	25–44 years	66 (50.7)	64 (49.3)	130 (28.1)
	45–59 years	11 (47.8)	12 (52.2)	23 (5.0)
	60 years and above	6 (40.0)	9 (60.0)	15 (3.2)
	Total	231 (100.0)	231 (100.0)	462(100.0)
Residence	Urban	157 (46.3)	182 (53.7)	339 (73.4)
	Rural	74 (60.2)	49 (39.8)	123 (26.6)
	Total	231 (100.0)	231 (100.0)	462 (100.0)
Religion	Orthodox	143 (48.3)	153 (51.7)	296 (64.1)
	Muslim	63 (51.6)	59 (48.4)	122 (26.4)
	Protestant	24 (63.2)	14 (36.8)	38 (8.2)
	Other	1 (16.7)	5 (83.3)	6 (1.3)
	Total	231(100.0)	231(100.0)	462 (100.0)
Occupation	Student	87 (45.5)	104 (54.5)	191 (41.3)
	Government employee	35 (46.1)	41 (53.9)	76 (16.5)
	Unemployed	51 (58.0)	37 (42.0)	88 (19.1)
	Farmer	28 (58.3)	20 (41.7)	48 (10.4)
	Other	30 (50.8)	29 (49.2)	59 (12.8)
	Total	231 (100.0)	231 (100.0)	462 (100.0)

Educational status	Illiterate	56(56.6)	43(43.4)	99 (21.4)
	Primary	61(48.8)	64(51.2)	125 (27.0)
	Secondary	61(51.3)	58(48.7)	119 (25.8)
	College and above	53(44.5)	66(55.5)	119 (25.8)
	Total	231 (100.0)	231(100.0)	462 (100.0)
Economic status	Low	168(50.8)	163(49.2)	331 (71.6)
	Moderate	48(49.5)	49(50.5)	97 (21.0)
	High	15(44.1)	19(55.9)	34 (7.4)
	Total	231 (100.0)	231 (100.0)	462 (100.0)
Marital status	Married	62 (49.2)	64 (50.8)	126 (27.3)
	Single	159 (50.5)	156 (49.5)	315 (68.2)
	Divorced	5 (45.5)	6 (54.5)	11 (2.4)
	Widowed	5 (50.0)	5 (50.0)	10 (2.1)
	Total	231 (100.0)	231 (100.0)	462 (100.0)
Health center	Han	50 (47.2)	56 (52.8)	106 (22.9)
	Shumabo	60 (48.4)	64 (51.6)	124 (26.8)
	Shimbit	87 (54.4)	73 (45.6)	160 (34.6)
	Addis Alem Hospital	34 (47.2)	38 (52.8)	72 (15.7)
	Total	231(100.0)	231 (100.0)	462 (100.0)
History of previous malaria infection	Yes	81 (63.3)	47 (36.7)	128 (27.7)
	No	150 (44.9)	184 (55.1)	334 (72.3)
	Total	231 (100.0)	231 (100.0)	462 (100.0)
Duration of illness	≤ 2 days	98 (45.0)	120 (55.0)	218 (47.2)
	3–5 days	76 (48.7)	80 (51.3)	156 (33.8)
	6–10 days	26 (60.5)	17 (39.5)	43 (9.3)
	>10 days	31 (68.9)	14 (31.1)	45 (9.7)
	Total	231 (100.0)	231 (100.0)	462 (100.0)

Key: Number (N), *Plasmodium falciparum* (*P. falciparum*), *Plasmodium vivax* (*P. vivax*).

6.2. The overall prevalence of hematological abnormalities among study participants

The most commonly encountered hematological abnormality among malaria-infected participants was thrombocytopenia, 265 (57.36%), of whom 132 (28.57%) had mild, 99 (21.43%) moderate, 30 (6.49%) severe, and 4 (0.87%) very severe thrombocytopenia (Fig. 3). The second most common abnormality was anemia, observed in 145 (31.39%), of whom 56 (12.12%) were mildly anemic, 54 (11.69%) moderately anemic, and 35 (7.58%) severely anemic (Fig. 4). Regarding WBC-related abnormalities, leukopenia was the most frequent 122 (26.4%), followed by neutropenia 114 (24.7%), and lymphopenia 97 (21%). Leukocytosis 25 (5.41%), neutrophilia 13 (2.81%), and lymphocytosis 10 (2.16%) were less frequently observed abnormalities.

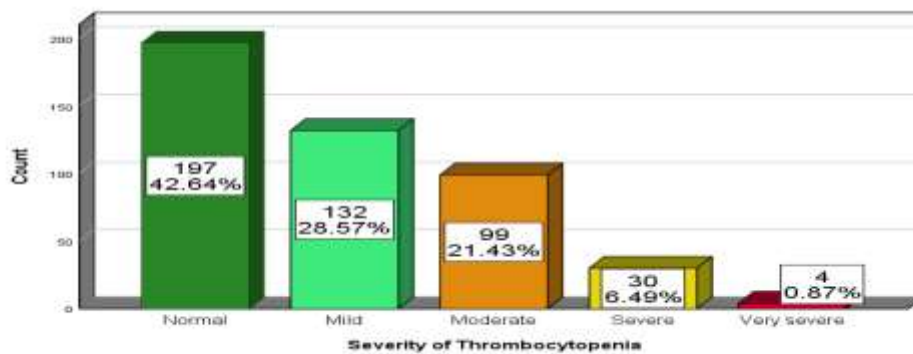


Figure 3: The prevalence and severity of thrombocytopenia among malaria-infected participants.

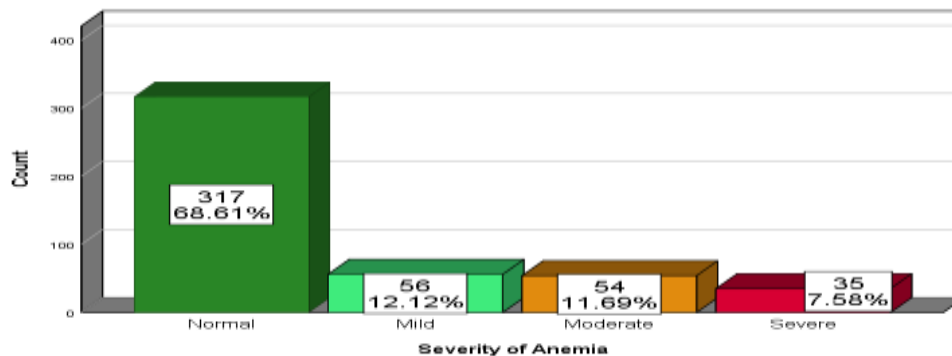


Figure 4: The prevalence and severity of anemia among malaria-infected study participants.

6.3. Comparison of hematological abnormalities between malaria species

The mean RBC counts were significantly higher among *P. vivax*-infected participants ($4.848 \pm 1.202 \times 10^{12}/L$) than among *P. falciparum*-infected participants ($4.536 \pm 1.292 \times 10^{12}/L$). Similarly, the total WBC counts among *P. vivax*-infected participants ($6.237 \pm 2.624 \times 10^9/L$) were significantly higher than those infected with *P. falciparum* ($5.431 \pm 2.472 \times 10^9/L$). In contrast, the mean RDW-CV was significantly higher in *P. falciparum*-infected participants ($14.401 \pm 2.210\%$) than those infected with *P. vivax* ($13.714 \pm 1.636\%$). However, there were no significant differences regarding MCV, MCH, and MCHC between the two malaria species ([Table 2](#)).

Table 2: Hematological parameters among study participants infected with *Plasmodium falciparum* and *Plasmodium vivax*.

Hematological parameters	Malaria species		Mean difference (95% CI)	p-value
	<i>P. vivax</i> (Mean $\pm$ SD)	<i>P. falciparum</i> (Mean $\pm$ SD)		
RBC counts ($\times 10^{12}/L$)	4.848 ± 1.202	4.536 ± 1.292	0.312 (0.083, 0.540)	0.008
HGB (g/dL)	14.723 ± 3.490	13.219 ± 3.834	1.504 (0.833, 2.174)	<0.001
HCT (%)	43.940 ± 9.589	40.093 ± 10.737	3.846 (1.985, 5.708)	<0.001
MCV (fL)	90.046 ± 10.53	88.730 ± 11.269	1.315 (-0.679, 3.309)	0.195
MCH (pg)	29.602 ± 3.039	29.246 ± 4.833	0.356 (-0.382, 1.094)	0.344
MCHC (g/dL)	33.376 ± 3.818	33.012 ± 4.114	0.364 (-0.362, 1.090)	0.325
RDW-CV (%)	13.714 ± 1.636	14.401 ± 2.210	-0.687 (-1.042, -0.331)	<0.001
WBC count ($\times 10^9/L$)	6.237 ± 2.624	5.431 ± 2.472	0.806 (0.340, 1.272)	0.001
Neutrophil ($\times 10^9/L$)	3.334 ± 1.779	2.813 ± 1.677	0.521 (0.205, 0.837)	0.001
Lymphocyte ($\times 10^9/L$)	1.946 ± 0.924	1.698 ± 0.868	0.248 (0.084, 0.412)	0.003
PLT count ($\times 10^9/L$)	187.42 ± 94.90	149.29 ± 91.973	38.13 (21.07, 55.243)	<0.001
MPV (fL)	9.520 ± 1.815	10.455 ± 2.140	-0.935 (-1.298, -0.572)	<0.001
PCT (%)	0.184 ± 0.074	0.152 ± 0.090	0.032 (0.017, 0.047)	<0.001
PDW (%)	14.842 ± 2.171	15.662 ± 2.832	-0.820 (-1.281, -0.359)	0.001

Key: Number (N), Probability value (p-value), Standard Deviation (SD), Confidence Interval (CI), *Plasmodium vivax* (*P. vivax*), Hemoglobin (HGB), *Plasmodium falciparum* (*P. falciparum*), Hematocrit (HCT), Mean Platelet Volume (MPV), Plateletcrit (PCT), Platelet Distribution Width (PDW), Platelet Distribution Width (PDW), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC).

The prevalence of thrombocytopenia was significantly higher among participants infected with *P. falciparum* (66.2%) than among those infected with *P. vivax* (48.5%). Similarly, the prevalence of anemia was significantly higher among *P. falciparum*-infected participants (41.1%) than among *P. vivax*-infected participants (21.6%). In addition, the prevalence of leukopenia was significantly higher among *P. falciparum*-infected participants (35.1%) than among those infected with *P. vivax* (17.7%). However, the prevalence of leukocytosis, neutrophilia, and lymphocytosis was higher among *P. vivax*-infected participants, although no significant differences were observed between the two malaria species ([Table 3](#)).

Table 3:-Hematological abnormalities between *Plasmodium falciparum* and *Plasmodium vivax* among study participants.

Hematological abnormalities	Malaria species		X ² -value	p-value
	<i>P. falciparum</i> N (%)	<i>P. vivax</i> N (%)		
Thrombocytopenia	153 (66.2)	112 (48.5)	14.876	< 0.001
Anemia	95 (41.1)	50 (21.6)	20.354	< 0.001
Leukopenia	81 (35.1)	41 (17.7)	17.821	< 0.001
Leukocytosis	10 (4.3)	15 (6.5)	1.057	0.304
Neutropenia	63 (27.3)	51 (22.1)	1.677	0.195
Neutrophilia	5 (2.2)	8 (3.5)	0.712	0.399
Lymphopenia	59 (25.5)	38 (16.5)	5.755	0.016
Lymphocytosis	4 (1.7)	6 (2.6)	0.409	0.523

Key: Number (N), Probability value (p-value), *Plasmodium vivax* (*P. vivax*), and *Plasmodium falciparum* (*P. falciparum*).

6.4. Determinants associated with hematological abnormalities

6.4.1. Determinants associated with anemia

Both bivariable and multivariable logistic regression analyses were performed to identify determinants of anemia among malaria-infected participants. In the bivariable analysis, associated factors with $p < 0.25$ were considered candidates for the multivariable model. Therefore, sex, age, residence, educational status, occupation, history of previous malaria infection, duration of illness, and malaria species were included in the multivariable analysis.

In the multivariable analysis, age, occupation, duration of illness, and malaria species were identified as determinants of anemia. Participants infected with *P. falciparum* had 3.476 times higher odds of developing anemia than those infected with *P. vivax* (AOR = 3.476, 95% CI: 1.838–6.574). Students had 9.703 times higher odds of developing anemia compared to other occupations (AOR = 9.703, 95% CI: 2.071–45.465). Those who were ill for ≤ 2 days had 99.1% lower odds of anemia than those who were ill more than 10 days (AOR = 0.009, 95% CI: 0.002–0.037). Regarding age, age 20–24 years was associated with reduced odds of anemia compared to those aged ≥ 60 years (AOR = 0.006, 95% CI: 0.000–0.099) ([Table 4](#)).

6.4.2. Determinants associated with thrombocytopenia

Similarly, for thrombocytopenia, sex, age, residence, educational status, occupation, history of previous malaria infection, duration of illness, and malaria species were candidates for multivariable analysis at $p < 0.25$. Only previous malaria infection, duration of illness, and malaria species were identified as determinants of thrombocytopenia. Participants with previous malaria history were 1.788-fold higher odds of developing thrombocytopenia compared to those who had no history (AOR = 1.788, 95% CI: 1.037–3.082).

Participants who were ill for ≤ 2 days were 73.1% lower odds of thrombocytopenia than those who were ill more than 10 days (AOR = 0.269, 95% CI: 0.093–0.779). Those who were ill between 3–5 days were 70.7% lower odds of thrombocytopenia than those who were ill more than 10 days (AOR = 0.293, 95% CI: 0.101–0.850). Participants infected with *P. falciparum* were 1.895 times more likely to develop thrombocytopenia than those infected with *P. vivax* (AOR = 1.895, 95% CI = 1.243–2.887) ([Table 5](#)).

6.4.3. Determinants associated with leukopenia

For leukopenia, age, residence, economic status, occupation, previous malaria infection, duration of illness, and malaria species were considered candidates for multivariable analysis ($p < 0.25$). Occupation, malaria species, duration of illness, and previous malaria infection were identified as determinants of leukopenia. For instance, the odds of being leukopenic among *P. falciparum*-infected participants were 2.214 times higher than those infected with *P. vivax* (AOR = 2.214, 95% CI: 1.369–3.580).

Participants with previous malaria infection had 2.782 times higher odds of leukopenia than those who did not have previous malaria history (AOR = 2.782, 95% CI: 1.619–4.780). Participants with duration of illness ≤ 2 days had 64.6% lower odds of neutropenia than those who were ill more than 10 days (AOR = 0.354, 95% CI: 0.150–0.836). Those who were ill between 3–5 days were 62.8% lower odds of neutropenia than those who were ill more than 10 days (AOR = 0.372, 95% CI: 0.158–0.877) ([Table 6](#)).

6.4.4. Determinants associated with neutropenia

In the bivariable logistic regression analysis, age, residence, previous malaria infection, duration of illness, and malaria species were significantly associated at $p < 0.25$ and considered candidates for multivariable analysis. In multivariable logistic regression, only duration of illness was identified as determinants of neutropenia. Participants who were ill for ≤ 2 days had 67.1% lower odds of neutropenia than those who were ill more than 10 days (AOR = 0.329, 95% CI: 0.149–0.729). Those who were ill between 3–5 days were 57.1% lower odds of neutropenia than those who were ill more than 10 days (AOR = 0.429, 95% CI: 0.195–0.946) ([Table 7](#)).

6.4.5. Determinates associated with lymphopenia

For lymphopenia, age, educational status, occupation, previous malaria infection, duration of illness, and malaria species were considered candidates for multivariable analysis ($p < 0.25$). Malaria species and education were identified as determinants of lymphopenia. *P. falciparum*-infected participants were 1.699 times more likely to develop lymphopenia than those infected with *P. vivax* (AOR = 1.699, 95% CI: 1.052–2.744). Illiterate participants were 6.126 times higher odds of lymphopenia than those who had college and above educational status (AOR = 6.126, 95% CI: 1.415–26.520) ([Table 8](#)).

Table 4:- Binary logistic regression analysis of determinants of anemia among malaria-infected study participants.

Variables	Categories	Anemic status		COR (95% CI)	p-value	AOR (95% CI)	p-value
		Yes N (%)	No N (%)				
Sex	Male	65 (25.7)	188 (74.3)	0.558 (0.375, 0.829)	0.004	0.657(0.352, 1.226)	0.187
	Female	80 (38.3)	129 (61.7)	1	---	1	---
Age	0–28 days	10 (90.9)	1 (9.1)	0.714 (0.040, 12.829)	0.819	0.431 (0.008, 23.610)	0.680
	29 days–12 months	21 (80.8)	5 (19.2)	0.300 (0.032, 2.849)	0.294	0.097 (0.003, 2.987)	0.182
	1–9 years	26 (33.8)	51 (66.2)	0.036 (0.005, 0.292)	0.002	0.011 (0.000, 0.270)	0.006
	10–19 years	27(23.6)	87 (76.4)	0.022 (0.003, 0.176)	<0.001	0.005 (0.000, 0.099)	<0.001
	20–24 years	15 (22.7)	51 (77.8)	0.021 (0.003, 0.173)	<0.001	0.006 (0.000, 0.099)	<0.001
	25–44 years	24 (18.5)	106 (81.5)	0.016 (0.002, 0.129)	<0.001	0.010 (0.001, 0.158)	0.001
	45–59 years	8 (34.8)	15 (65.2)	0.038 (0.004, 0.345)	0.004	0.021 (0.001, 0.402)	0.010
	≥ 60 years	14 (93.3)	1 (6.7)	1	---	1	---
Residence	Urban	89 (26.3)	250 (73.7)	0.426 (0.277, 0.654)	<0.001	0.552 (0.243, 1.257)	0.157
	Rural	56(45.5)	67 (54.5)	1	---	1	---
Educational status	Illiterate	54 (54.5)	45 (45.5)	3.560 (2.008, 6.311)	<0.001	0.547 (0.095, 3.140)	0.499
	Primary	29 (23.2)	96 (76.8)	0.896 (0.449, 1.611)	0.714	1.117 (0.323, 3.866)	0.862
	Secondary	32 (26.8)	87 (73.2)	1.091 (0.611, 1.947)	0.768	1.103 (0.377, 3.230)	0.858
	College and above	30 (25.2)	89 (74.8)	1	---	1	---
Occupation	Student	51 (26.7)	140 (73.3)	1.427 (0.701, 2.903)	0.327	9.703 (2.071, 45.465)	0.004
	Government employee	18 (23.7)	58 (76.3)	1.216 (0.532, 2.775)	0.643	2.638 (0.537, 12.953)	0.232
	Unemployed	49 (55.7)	39 (44.3)	4.921 (2.300, 10.530)	<0.001	8.247 (0.961, 70.802)	0.054
	Farmer	15 (31.3)	33 (68.7)	1.780 (0.738, 4.292)	0.199	2.115 (0.432, 10.353)	0.355
	Other	12 (20.3)	47 (79.7)	1	---	1	---
Economic status	Low	111 (33.5)	220 (66.5)	1.402 (0.633, 3.105)	0.405	---	---
	Medium	25 (28.3)	72 (74.2)	0.965 (0.397, 2.343)	0.936	---	---
	High	9 (26.5)	25 (73.5)	1	---	---	---
Previous malaria infection	Yes	75 (58.6)	53 (41.4)	5.337 (3.439, 8.282)	<0.001	2.019 (0.999, 4.082)	0.050
	No	70 (21.0)	264 (79.0)	1	---	1	---
Duration of illness	≤ 2 days	14 (6.4)	204 (93.6)	0.007 (0.002, 0.021)	<0.001	0.009(0.002, 0.037)	<0.001
	3–5 days	52 (33.3)	104 (66.7)	0.049 (0.017, 0.144)	<0.001	0.062 (0.017, 0.226)	<0.001
	6–10 days	38 (88.4)	5 (11.6)	0.741 (0.185, 2.968)	0.672	0.675 (0.139, 3.279)	0.626
	> 10 days	41 (91.1)	4 (8.9)	1	---	1	---
Malaria species	<i>P.falciparum</i>	95(41.1)	136 (58.9)	2.529 (1.681, 3.804)	<0.001	3.476 (1.838, 6.574)	<0.001
	<i>P.vivax</i>	50 (21.6)	181 (78.4)	1	---	1	---

KEY: Number (N), *Plasmodium falciparum* (*P. falciparum*), *Plasmodium vivax* (*P. vivax*), Crude Odds Ratio (COR), Probability value (p-value), Adjusted Odds Ratio (AOR), and Confidence Interval (CI).

Table 5:- Binary logistic regression analysis of determinants of thrombocytopenia among malaria-infected study participants.

Variables	Categories	Thrombocytopenia status		COR (95% CI)	p-value	AOR (95% CI)	p-value
		Yes N (%)	No N (%)				
Sex	Male	133 (52.6)	120 (47.4)	0.647 (0.455, 0.940)	0.022	0.738 (0.479, 1.136)	0.167
	Female	132 (63.2)	77 (36.8)	1	---	1	---
Age	0–28 days	10 (90.9)	1 (9.1)	1.538 (0.122, 19.470)	0.739	2.234 (0.115, 43.446)	0.596
	29 days–12 months	25 (96.2)	1 (3.8)	3.846 (0.318, 46.494)	0.289	5.509 (0.310, 97.910)	0.245
	1–9 years	49 (63.6)	28 (36.4)	0.269 (0.057, 1.280)	0.099	0.668 (0.091, 4.907)	0.692
	10–19 years	52 (45.6)	62(54.4)	0.129 (0.028, 0.598)	0.009	0.368 (0.057, 2.362)	0.292
	20–24 years	30 (45.5)	36 (54.5)	0.128 (0.027, 0.614)	0.010	0.260(0.046, 1.471)	0.128
	25–44 years	72(55.4)	58 (44.6)	0.191 (0.041, 0.881)	0.034	0.384(0.074, 1.992)	0.255
	45–59 years	14 (60.9)	9 (39.1)	0.239 (0.043, 1.321)	0.101	0.292(0.046, 1.863)	0.193
	≥ 60 years	13 (86.7)	2 (13.3)	1	---	1	---
Residence	Urban	182 (53.7)	157 (46.3)	0.559 (0.362, 0.862)	0.008	0.884 (0.468, 1.671)	0.705
	Rural	83 (67.5)	40 (32.5)	1	---	1	---
Educational status	Illiterate	81 (81.8)	18 (18.2)	4.000 (2.141, 7.472)	<0.001	2.165 (0.589, 7.959)	0.245
	Primary	61 (48.8)	64 (51.2)	0.847 (0.513, 1.400)	0.518	0.676 (0.326, 1.402)	0.293
	Secondary	60 (50.4)	59 (49.6)	0.904 (0.544, 1.503)	0.697	0.830 (0.426, 1.619)	0.585
	College and above	63 (52.9)	56 (47.1)	1	---	1	---
Economic status	Low	191 (57.7)	140 (42.3)	1.213 (0.597, 2.461)	0.593	---	---
	Medium	56 (57.7)	41 (42.3)	1.214 (0.554, 2.661)	0.628	---	---
	High	18 (52.9)	16 (47.1)	1	---	1	---
Occupation	Student	90 (47.1)	101 (52.9)	0.702 (0.390, 1.263)	0.238	0.847 (0.352, 2.034)	0.710
	Government employee	42 (55.3)	34 (44.7)	0.973 (0.491, 1.930)	0.938	1.109 (0.491, 2.505)	0.804
	Unemployed	68 (77.3)	20 (22.7)	2.679 (1.309, 5.482)	0.007	0.381 (0.089,1.633)	0.194
	Farmer	32 (66.7)	16 (33.3)	1.576 (0.715, 3.472)	0.259	1.251 (0.441, 3.546)	0.673
	Other	33 (55.9)	26 (44.1)	1	---	1	---
Previous malaria infection	Yes	97 (75.8)	31 (24.2)	3.092 (1.956, 4.887)	<0.001	1.788 (1.037, 3.082)	0.037
	No	168 (50.3)	166 (49.7)	1	---	1	---
Duration of illness	≤ 2 days	102 (46.8)	116 (53.2)	0.110 (0.042, 0.289)	<0.001	0.269 (0.093, 0.779)	0.016
	3–5 days	84 (53.8)	72 (46.2)	0.146 (0.055, 0.389)	<0.001	0.293 (0.101, 0.850)	0.024
	6–10 days	39 (90.7)	4 (9.3)	1.219 (0.305, 4.877)	0.780	1.471 (0.341, 6.339)	0.605
	> 10 days	40 (88.9)	5 (11.1)	1	---	1	---
Malaria species	<i>P.falciparum</i>	153 (66.2)	78 (33.8)	2.084 (1.432, 3.034)	<0.001	1.895 (1.243, 2.887)	0.003
	<i>P.vivax</i>	112 (48.5)	119 (51.5)	1	---	1	---

KEY: Number (N), *Plasmodium falciparum* (*P. falciparum*), *Plasmodium vivax* (*P. vivax*), Crude Odds Ratio (COR), Probability value (p-value), Adjusted Odds Ratio (AOR), and Confidence Interval (CI).

Table 6:- Binary logistic regression analysis of determinants of leukopenia among malaria-infected study participants.

Variables	Categories	Leukopenia		COR (95% CI)	p-value	AOR (95% CI)	p-value
		Yes N (%)	No N (%)				
Sex	Male	71 (28.1)	182 (71.9)	1.209 (0.796, 1.836)	0.375	---	---
	Female	51 (24.4)	158 (75.6)	1	---	1	---
Age	0–28 days	2 (18.2)	9 (81.8)	0.444 (0.068, 2.886)	0.396	1.034 (0.085, 12.617)	0.979
	29 days–12 months	5 (19.2)	21 (80.8)	0.476 (0.112, 2.031)	0.316	1.284 (0.163, 10.118)	0.812
	1–9 years	13 (16.9)	64 (83.1)	0.406 (0.119, 1.387)	0.151	1.189(0.206,6.860)	0.846
	10–19 years	26 (22.8)	88 (77.2)	0.591(0.185, 1.884)	0.374	1.001 (0.198, 5.066)	0.999
	20–24 years	17 (25.8)	49 (74.2)	0.694(0.208, 2.320)	0.553	0.998 (0.217, 4.590)	0.998
	25–44 years	43 (33.1)	87 (66.9)	0.989 (0.318, 3.072)	0.984	1.773 (0.447, 7.036)	0.415
	45–59 years	11 (47.8)	12 (52.2)	1.833 (0.475, 7.070)	0.379	2.596 (0.536, 12.573)	0.236
	≥ 60 years	5 (33.3)	10 (66.7)	1	---	1	---
Residence	Urban	74 (21.8)	265 (78.2)	0.436(0.280, 0.681)	<0.001	0.954(0.494, 1.842)	0.888
	Rural	48 (39.0)	75 (61.0)	1	---	1	---
Educational status	Illiterate	24 (24.0)	76 (76.0)	0.789(0.426, 1.459)	0.450	---	----
	Primary	32 (24.2)	100 (75.8)	0.823 (0.464, 1.461)	0.506	---	---
	Secondary	36 (30.0)	84 (70.0)	1.130(0.645, 1.980)	0.668	---	---
	College and above	30 (27.3)	80 (72.7)	1	---	1	---
Economic status	Low	80 (24.2)	251 (75.8)	1.229(0.516, 2.930)	0.614	2.051(0.644, 6.534)	0.224
	Medium	35 (36.1)	62 (63.9)	2.177(0.860, 5.512)	0.101	1.650 (0.570, 4.779)	0.356
	High	7 (20.6)	27 (79.4)	1	---	1	---
Occupation	Student	46 (24.1)	145 (75.9)	0.574 (0.306, 1.075)	0.083	0.676 (0.256, 1.791)	0.431
	Government employee	20 (26.3)	56 (73.7)	0.646 (0.309, 1.352)	0.246	0.741 (0.323, 1.703)	0.480
	Unemployed	13 (14.8)	75 (85.2)	0.314 (0.142, 0.694)	0.004	0.149 (0.035, 0.638)	0.010
	Farmer	22 (45.8)	26 (54.2)	1.531 (0.703,3.336)	0.284	1.142 (0.429, 3.040)	0.790
	Other	21(35.6)	38 (64.4)	1	---	1	---
Previous malaria infection	Yes	59 (46.1)	69 (53.9)	3.678 (2.363, 5.726)	<0.001	2.782(1.619, 4.780)	<0.001
	No	63 (18.9)	271 (81.1)	1	---	1	---
Duration of illness	≤ 2 days	45 (20.6)	173 (79.4)	0.249 (0.127, 0.486)	<0.001	0.354(0.150, 0.836)	0.018
	3–5 days	33 (21.2)	123 (78.8)	0.257 (0.128, 0.517)	<0.001	0.372(0.158, 0.877)	0.024
	6–10 days	21 (48.8)	22 (51.2)	0.913 (0.396, 2.107)	0.831	1.418 (0.534, 3.766)	0.483
	> 10 days	23 (51.1)	22 (48.9)	1	---	1	---
Malaria species	<i>P.falciparum</i>	81 (35.1)	150 (64.9)	2.502 (1.624, 3.856)	<0.001	2.214(1.369, 3.580)	0.001
	<i>P.vivax</i>	41 (17.7)	190 (82.3)	1	---	1	---

KEY: Number (N), *Plasmodium falciparum* (*P. falciparum*), *Plasmodium vivax* (*P. vivax*), Crude Odds Ratio (COR), Probability value (p-value), Adjusted Odds Ratio (AOR), and Confidence Interval (CI).

Table 7:- Binary logistic regression analysis of determinants of neutropenia among malaria-infected study participants.

Variables	Categories	Neutropenia		COR (95% CI)	p-value	AOR (95% CI)	p-value
		Yes N (%)	No N (%)				
Sex	Male	61 (24.1)	192 (75.9)	0.935 (0.612, 1.429)	0.757	---	---
	Female	53 (25.4)	156 (74.6)	1	---	1	---
Age	0–28 days	4 (36.4)	7 (63.6)	8.000 (0.747, 85.725)	0.086	9.230 (0.829, 102.778)	0.071
	29 days–12 months	8 (30.8)	18 (69.2)	6.222 (0.694, 55.766)	0.102	6.221 (0.678, 57.081)	0.106
	1–9 years	19 (24.7)	58 (75.3)	4.586 (0.565, 37.223)	0.154	6.572 (0.779, 55.461)	0.084
	10–19 years	24 (21.1)	90 (78.9)	3.733 (0.467, 29.827)	0.214	5.643 (0.675, 47.251)	0.110
	20–24 years	17 (25.8)	49 (74.2)	4.857 (0.593, 39.761)	0.141	7.087 (0.832, 60.404)	0.073
	25–44 years	35 (26.9)	95 (73.1)	5.158 (0.654, 40.689)	0.120	7.679 (0.929, 63.438)	0.058
	45–59 years	6 (26.1)	17 (73.9)	4.941 (0.530, 46.047)	0.161	6.229 (0.642, 60.473)	0.115
	≥ 60 years	1 (6.7)	14 (93.3)	1	---	1	---
Residence	Urban	76 (22.4)	263 (77.6)	0.646 (0.408, 1.024)	0.063	0.805 (0.489, 1.324)	0.393
	Rural	38 (30.9)	85 (69.1)	1	---	1	---
Educational status	Illiterate	26 (26.3)	73 (73.7)	1.274 (0.683, 2.378)	0.447	---	---
	Primary	35 (28.0)	90 (72.0)	1.391 (0.775, 2.495)	0.268	---	---
	Secondary	27 (22.7)	92 (77.3)	1.050 (0.570, 1.934)	0.876	---	---
	College and above	26 (21.8)	93 (78.2)	1	---	1	---
Economic status	Low	81 (24.5)	250 (75.5)	1.250 (0.524, 2.978)	0.615	---	---
	Medium	26 (26.8)	71 (73.2)	1.412 (0.549, 3.634)	0.474	---	---
	High	7 (20.6)	27 (79.4)	1	---	1	---
Occupation	Student	41 (21.5)	150 (78.5)	0.735 (0.376, 1.435)	0.367	---	---
	Government employee	20 (26.3)	56 (73.7)	0.960 (0.445, 2.069)	0.917	---	---
	Unemployed	23 (26.1)	65 (73.9)	0.951 (0.451, 2.004)	0.895	---	---
	Farmer	14 (29.2)	34 (70.8)	1.107 (0.475, 2.580)	0.815	---	---
	Other	16 (27.1)	43 (72.7)	1	---	1	---
Previous malaria infection	Yes	38 (29.7)	90 (70.3)	1.433 (0.907, 2.265)	0.123	0.959 (0.556, 1.654)	0.880
	No	76 (22.8)	258 (77.2)	1	---	1	---
Duration of illness	≤ 2 days	44 (20.2)	174 (79.8)	0.346 (0.176, 0.618)	0.002	0.329 (0.149, 0.729)	0.006
	3–5 days	38 (24.4)	118 (75.6)	0.441 (0.220, 0.883)	0.021	0.429 (0.195, 0.946)	0.036
	6–10 days	13 (30.2)	30 (69.8)	0.593 (0.246, 1.429)	0.244	0.597 (0.239, 1.495)	0.271
	> 10 days	19 (42.2)	26 (57.8)	1	---	1	---
Malaria species	<i>P.falciparum</i>	63 (27.3)	168 (72.7)	1.324 (0.865, 2.024)	0.196	1.150 (0.737, 1.792)	0.539
	<i>P.vivax</i>	51 (22.1)	180 (77.9)	1	---	1	---

KEY: Number (N), *Plasmodium falciparum* (*P. falciparum*), *Plasmodium vivax* (*P. vivax*), Crude Odds Ratio (COR), Probability value (p-value), Adjusted Odds Ratio (AOR), and Confidence Interval (CI).

Table 8:- Binary logistic regression analysis of determinants of lymphopenia among malaria-infected study participants.

Variables	Categories	Lymphopenia		COR (95% CI)	p-value	AOR (95% CI)	p-value
		Yes N (%)	No N (%)				
Sex	Male	53 (20.9)	200 (79.1)	0.994 (0.634, 1.558)	0.978	---	---
	Female	44 (21.1)	165 (78.9)	1	---	1	---
Age	0–28 days	2 (18.2)	9 (81.8)	0.333 (0.053, 2.155)	0.244	0.296 (0.026, 3.324)	0.324
	29 days–12 months	9 (34.6)	17 (65.4)	0.794 (0.214, 2.947)	0.730	0.599 (0.080, 4.475)	0.618
	1–9 years	18 (23.4)	59 (76.3)	0.458 (0.143, 1.460)	0.187	0.394 (0.066, 2.344)	0.306
	10–19 years	23 (20.2)	91 (79.8)	0.379 (0.123, 1.173)	0.092	0.373 (0.076, 1.830)	0.225
	20–24 years	10 (15.2)	56 (84.8)	0.268 (0.078, 0.919)	0.036	0.339 (0.082, 1.396)	0.134
	25–44 years	24 (18.5)	106 (81.5)	0.340 (0.110, 1.045)	0.060	0.426 (0.125, 1.449)	0.172
	45–59 years	5 (21.7)	18 (78.3)	0.417 (0.100, 1.743)	0.231	0.427 (0.094, 1.937)	0.270
	≥ 60 years	6 (40.0)	9 (60.0)	1	---	1	---
Residence	Urban	69 (20.4)	270 (79.6)	0.867 (0.527, 1.426)	0.574	---	---
	Rural	28 (22.8)	95 (77.2)	1	---	1	---
Educational status	Illiterate	28 (28.3)	71 (71.7)	1.646 (0.876, 3.094)	0.122	6.126 (1.415, 26.520)	0.015
	Primary	25 (20.0)	100 (80.0)	1.043 (0.555, 1.963)	0.895	1.612 (0.680, 3.819)	0.278
	Secondary	21 (17.6)	98 (82.4)	0.894 (0.465, 1.722)	0.738	1.248 (0.549, 2.837)	0.597
	College and above	23 (19.3)	96 (80.7)	1	---	1	---
Economic status	Low	69 (20.8)	262 (79.2)	1.527 (0.570, 4.092)	0.399	---	---
	Medium	23 (23.7)	74 (76.3)	1.803 (0.626, 5.194)	0.275	---	---
	High	5 (14.7)	29 (85.3)	1	---	1	---
Occupation	Student	38 (19.9)	153 (80.1)	1.217 (0.565, 2.621)	0.616	1.647 (0.552, 4.912)	0.371
	Government employee	20 (26.3)	56 (73.7)	1.750 (0.748, 4.096)	0.197	2.403 (0.919, 6.287)	0.074
	Unemployed	22 (25)	66 (75)	1.633 (0.709, 3.760)	0.249	0.377 (0.057, 2.474)	0.310
	Farmer	7 (14.6)	41 (85.4)	0.837 (0.292, 2.394)	0.739	0.362 (0.095, 1.378)	0.136
	Other	10 (16.9)	49 (83.1)	1	---	1	---
Previous malaria infection	Yes	33 (25.8)	95 (74.2)	1.465 (0.906, 2.370)	0.119	1.118 (0.633, 1.972)	0.701
	No	64 (19.2)	270 (80.8)	1	---	1	---
Duration of illness	≤ 2 days	37 (17.0)	181 (83.0)	0.371 (0.183, 0.750)	0.006	0.537 (0.234, 1.231)	0.142
	3–5 days	32 (20.5)	124 (79.5)	0.468 (0.227, 0.964)	0.040	0.635 (0.281, 1.435)	0.275
	6–10 days	12 (27.9)	31 (72.1)	0.702 (0.284, 1.732)	0.442	0.785 (0.304, 2.026)	0.617
	> 10 days	16 (35.6)	29 (64.4)	1	---	1	---
Malaria species	<i>P.falciparum</i>	59 (25.5)	172 (74.5)	1.742 (1.104, 2.750)	0.017	1.699 (1.052, 2.744)	0.030
	<i>P.vivax</i>	38 (16.5)	193 (83.5)	1	---	1	---

KEY: Number (N), *Plasmodium falciparum* (*P. falciparum*), *Plasmodium vivax* (*P. vivax*), Crude Odds Ratio (COR), Probability value (p-value), Adjusted Odds Ratio (AOR), and Confidence Interval (CI).

7. Discussion

Malaria is a vector-borne infectious disease that causes significant variations in blood cell counts (8). Anemia, thrombocytopenia, and leukopenia are among the common hematological abnormalities associated with malaria infection (19). In this study, a total of 462 participants were included to assess the overall prevalence of these abnormalities. The prevalence of thrombocytopenia in the present study (57.36%) was lower than those reported in India (70%) (21), the UAE (83%) (22), Morocco (84%) (23), and Ethiopia (67.7%) (26), but it is comparable to a study conducted in Pakistan (58%) (27). The lower prevalence observed in the current study compared to these studies may be due to differences in study populations, parasite density, duration of illness, and host immunity across studies (72).

Anemia was the second most common hematological abnormality, accounting for (31.4%) of cases. This prevalence is lower than those reported in India (94%) (21), the UAE (64%) (22), Morocco (90%) (23), Tanzania (61%) (24), Nigeria (38.6%) (25), and Harar, Ethiopia (47%) (26). The lower occurrence in this study may be due to variations in malaria species distribution, host immunity, parasite density, nutritional status, and timing of presentation. Moreover, differences in the extent of hemolysis, and iron status and bone marrow suppression among participants may further explain these variations (73).

Regarding WBC-related abnormalities, the prevalence of leukopenia in the current study was 26.41%, which is higher than that reported in Colombia (18%) (28), but lower than that reported in Harar, Ethiopia (31.9%) (26). The higher prevalence compared with the Colombian study may be related to differences in host immune status and disease severity. In contrast, the lower prevalence compared with the Harar study may be explained by differences in parasite density, duration of illness, and study population characteristics.

Similarly, the prevalence of lymphopenia in this study was 21%, which is higher than that reported in India (12%) (21), but lower than that reported in the UAE (24%) (22) and Morocco (78%) (23). This lower prevalence compared to these studies may be due to variations in host immune status, parasite concentration, and disease severity. In addition, the prevalence of neutropenia was 24.7%, which was higher than that reported in the Netherlands (8%) (29). This difference may be related to variations in host immune status, parasite concentration, and disease severity, which can affect neutrophil production, utilization, and peripheral distribution (22).

In addition, 5.4% of participants in the current study developed leukocytosis, which was lower than that reported in Harar, Ethiopia (13%)(26). Similarly the prevalence of neutrophilia in this study was 2.8%, which was lower than that reported in the Netherlands (4%) (29). This the lower prevalence leukocytosis and neutrophilia compared to other studies might be because of differences in laboratory cut-off values, co-existing infections, disease severity, nutritional status and host immunity across the study populations(22).

In species-specific analysis, the prevalence of anemia in the current study among *P. falciparum*-infected participants (41.1%) was significantly higher than participants infected with *P. vivax* (21.6%). Anemia among *P. falciparum*-infected participants in this study was lower than findings reported in Ghana (67%) (33), in Cameroon (63.64%) (34), in Sudan (60.4%) (35), and in Dawuro, Ethiopia (65.7%)(36). This lower prevalence may be due to variation in host immune response and parasite density. In contrast the prevalence was higher than those from Raya Alamata, Ethiopia (34%)(37), this may be due to difference in duration of illness.

Regarding *P. vivax*, the frequency of anemia was comparable with that reported in Indonesia (21%) (30) and Mendi town, Ethiopia (29.9%)(46), but lower than that reported in Brazil (55.3%) (31) and Ethiopia (52.4%)(36). This lower prevalence compared to these studies may be related to variation in duration of illness and disease severity. The higher occurrence of anemia among *P. falciparum* than *P. vivax* in the current study may be related to the biological characteristics of the *P. falciparum* parasite, including its ability to infect all age groups of RBCs, multiple parasites can present in a single RBC, rapid multiplication, enhanced cytoadherence, and increased sequestration of infected RBCs (74).

Similarly, in this study, the prevalence of thrombocytopenia among *P. falciparum*-infected participants (66.2%) was significantly higher than those infected with *P. vivax* (48.5%). The prevalence of thrombocytopenia among *P. falciparum*-infected participants in the current study was higher than findings in Pakistan (26%) (39), Ghana (47.5%)(32), Cameroon (14.9%)(41), and Raya Alamata Hospital, Ethiopia (46.3%) (37). This higher prevalence may be as a result of differences in parasite density, disease severity, and study population characteristics. However lower than that reported in India (72%)(38), Saudi Arabia (96%)(40), and Dawuro, Ethiopia (83%) (36), this difference may be due to disease severity and duration of illness.

The occurrence of thrombocytopenia among *P. vivax*-infected participants in this study was lower than that reported in Pakistan (55.4%)(39), Venezuela (58.97%)(44), Saudi Arabia (86.8%)(40), and Dawuro, Ethiopia (85%)(36). The lower prevalence compared with these studies may be explained by differences in host immune status, parasite density, and disease severity. In contrast, the prevalence was higher than reports in Colombia (10.9%)(47), and Raya Alamata Hospital, Ethiopia (40.3%)(37). This higher prevalence may be related to differences in illness duration, disease severity, and study population characteristics. The higher incidence of thrombocytopenia among *P. falciparum* cases compared to *P. vivax* in the present study may be linked to the biological mechanism of *P. falciparum* infection, including increased splenic sequestration and greater bone marrow suppression(53, 75).

Similarly, in the present study the prevalence of leukopenia among *P. falciparum*-infected participants (35.1%) was significantly higher than those infected with *P. vivax* (17.7%). The prevalence of leukopenia among *P. falciparum*-infected participants was lower than that reported from India (42%) (43), Cameroon (94.9%)(34), and Ethiopia (74.1%) (37). This lower prevalence compared to these studies may be due to difference in study population characteristics and illness duration. In contrast, the prevalence was higher than that reported in Sudan (12.5%) (35). This higher prevalence may be related to differences in study population characteristics. Leukopenia among *P. vivax* was nearly comparable with findings reported from Pakistan (21.2%) (52), the UAE (14%) (22), and Ethiopia (15.5%) (37) but lower than those from Indonesia (45.9%) (50) and higher than those from Venezuela (4.5%) (49), this discrepancy may be related to differences in disease severity.

Furthermore, neutropenia among *P. falciparum*-infected participants (27.3%) was comparable with findings reported in Ghana (29.24%) (32) and Sudan (26.6%)(35). However, neutropenia among *P. vivax*-infected participants (22.1%) was higher than reports from Indonesia (50) and Venezuela(49). The higher prevalence of neutropenia compared to these studies may be due to differences in population characteristics. In addition, lymphopenia among *P. falciparum*-infected participants (25.5%) was lower than that reported in Ghana (32) and Ethiopia (36), but higher than findings from Sudan (5.7%)(35), this lower prevalence of lymphopenia compared to these studies may be due to variation in the level of parasitemia across the studies.

Among *P. vivax*-infected participants, lymphopenia (16.5%) was lower than reports from Indonesia (50), the UAE(22), and Ethiopia (36), but comparable to findings from Venezuela (49). The increased occurrence of leukopenia, neutropenia, and lymphopenia among *P. falciparum*-infected participants compared with *P. vivax*-infected participants in the current study may be due to its enhanced cytoadherence and sequestration of infected cells, greater bone marrow suppression, and higher levels of parasitemia(8, 28).

In contrast, leukocytosis, neutrophilia, and lymphocytosis in the current study were more prevalent among *P. vivax*-infected participants than those infected with *P. falciparum*. The prevalence of neutrophilia among *P. falciparum*-infected participants in the current study (2.2%) was lower than findings in Ghana (10.6%) (32) and Ethiopia (21%) (36). Similarly, the prevalence of leukocytosis the present study (4.3%) was also lower than findings in Ghana (32), but comparable with reports from Ethiopia (37).

Concerning *P. vivax*, the incidence of neutrophilia in the current study (3.5%) was comparable to a report from Indonesia (2.70%) (50) but lower than a study from Ethiopia (37.7%) (36). Likewise, leukocytosis in the present study (6.5%) was lower than reports from India (51) and Pakistan (52), but higher than findings from the UAE(22) and Ethiopia (37). In addition, the finding of lymphocytosis in the current study (2.6%) was comparable with findings from Indonesia (50). The increased occurrence of leukocytosis, neutrophilia, and lymphocytosis among *P. vivax*-infected participants compared with *P. falciparum*-infected participants in the current study may be due to nutritional status, immunity of participant, and the presence or absence of co-existing infection among participants(22).

Regarding determinants, female participants in the current study were more likely to have thrombocytopenia than males, and the difference was not statistically significant ($p = 0.167$). This finding is consistent with those reported in Pakistan ($p = 0.320$) (53) and Rwanda ($p = 0.689$) (54), where no significant association was observed. Similarly, no significant association was observed between thrombocytopenia and other demographic factors, which align with findings from Harar, Ethiopia(26). The lack of association may be due to the influence of unmeasured confounding variables.

Female participants in this study were also more anemic than their counterparts; however, the association was not statistically significant ($p = 0.187$), which is inconsistent with findings reported from Uganda (55) but consistent with a study conducted in Harar, Ethiopia (26). The higher prevalence of anemia among females may be by reason of menstrual blood loss and nutritional deficiency during reproductive age (76). Similarly, no significant association was observed between anemia and other socio-demographic factors, which is consistent with findings from Harar, Ethiopia (26).

Concerning Age, in this study, although a higher proportion of thrombocytopenia was observed among children and older participants, the association was not statistically significant. This finding is consistent with a report from Harar, Ethiopia (26), but differs from the Rwandan study, where children under five years showed significant association (AOR = 5.17, 95% CI: 1.674–16.259, $p = 0.004$) (54). The absence of significant association in this study may be due to the influence of unmeasured confounding variables.

Moreover, participants aged 1–9, 10–19, 20–24, 25–44, and 45–59 years showed a statistically significant association with anemia; however, a higher proportion of anemia was observed among extreme age groups. This finding differs from findings reported in Nigeria (56) and Thailand (57), where children under-five showed a significant association. This higher proportion of anemia among extreme ages may be due to increased nutritional deficiency and weaker immune status (77). The lack of significant association among under-five children in this study may be explained by the influence of unmeasured confounding variables.

Regarding clinical factors, participants with a short duration of illness (<5 days) are significantly less likely to develop anemia than those with a prolonged duration, which is consistent with findings in Thailand (57). Similarly, participants with a short duration of illness are also less likely to develop leukopenia, in line with reports from Thailand (58). This lower prevalence of anemia and leukopenia among participants with short duration of illness may be related to low parasite burden and less bone marrow suppression than prolonged duration. Moreover, participants with previous history of malaria are more likely to develop anemia and thrombocytopenia, consistent with findings reported in Indonesia (59).

8. Strength and limitations

The study addressed all major hematological abnormalities including anemia, thrombocytopenia, and leukopenia. In addition, this study provided species-specific comparison between *P. falciparum* and *P. vivax* and employed multivariable analysis for each hematological abnormalities. It also encompassed a wide age range of study participants and assessed many clinical and socio-demographic determinants, improving the comprehensiveness of the findings.

Conversely, this study has some limitations. The cross-sectional design of the study prevents establishing causality or temporal changes in hematological abnormalities and their determinants. Potential confounders that can affect hematological parameters such as chronic infections, co-existing infections (bacterial or viral), or genetic hematological disorders were not confirmed using laboratory methods. Likewise, more sensitive diagnostic methods, such as polymerase chain reaction (PCR) for malaria species detection and Kato-Katz for stool parasite identification were not used. Moreover, female participants of reproductive age were not confirmed using laboratory testing for human chorionic gonadotropin (hCG).

In addition, parasite load was not quantitatively assessed, which limited the evaluation of its potential relationship with hematological abnormalities. Nutritional status was also not assessed, although nutritional deficiencies particularly iron, folate, and vitamin B12 deficiencies, may influence hematological parameters. Economic status was assessed through face-to-face interviews using criteria adapted from previous studies; however, it was not objectively verified through observation.

9. Conclusion

This study indicates that malaria infection is associated with hematological abnormalities, mainly anemia, thrombocytopenia, leukopenia, neutropenia, and lymphopenia, telling that malaria significantly contributes to impaired hematological function. The more pronounced abnormalities observed among *P. falciparum*-infected patients imply that malaria species plays an important role in disease severity and patient outcomes, with *P. falciparum* infection demonstrating a higher-risk condition. Furthermore, the observed associations between hematological abnormalities and determinant factors such as prolonged illness duration, previous malaria infection, older adults, and malaria species suggest that delayed health-seeking behavior and susceptibility among certain population groups aggravates disease impact. Overall, these findings emphasize the need for early diagnosis, swift treatment, and routine assessment of hematological parameters during patient evaluation to reduce hematological complications and improve the clinical management of malaria-infected patients in the study area.

10.Recommendation

It is recommended that simple, inexpensive, and readily available hematological tests (CBC) should be used routine in the management of all malaria-infected patients in health facilities. Clinicians should also consider malaria species during patient management, as *P. falciparum* infection was more severe and significantly associated with higher odds of multiple hematological abnormalities compared to *P. vivax*. In addition, early diagnosis and rapid treatment of malaria should be emphasized to minimize complications, since longer duration of illness (>10 days) was strongly associated with increased risk of hematological abnormalities.

Special attention and close monitoring should be given to high-risk groups, including older adults (≥ 60 years), uneducated individuals and patients with previous history of malaria infection. Furthermore, all health institutions should integrate key determinant factors (such as duration of illness, malaria species, and malaria history) into routine clinical assessment for effective management of hematological abnormalities among malaria-infected patients. In addition, other researchers should conduct longitudinal studies to establish causal relationships between malaria infection and hematological abnormalities and investigate underlying biological mechanisms.

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12. Annex

Annex 1: Information sheet (English version)

Part 1: Introduction

This research entitled, “Hematological Abnormalities and Their Determinants among Patients Infected with *Plasmodium falciparum* and *Plasmodium vivax* in Bahir Dar, Northwest Ethiopia: A Cross-Sectional Study,” is being conducted in partial fulfillment of the requirements for the Master’s degree in Clinical Hematology and Immunohematology.

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Part 2: Invitation to take part in the study

You are invited to take part in this research study. Before you decide to participate, it is important for you to understand why the research is being conducted. This Participant Information Sheet tells you about the purpose, risks, and benefits of this research study. If you agree to participate, you will be asked to sign a consent form. If there is anything that you are not clear about, the researcher will explain it to you. Please take as much time as you need to read it. You should only consent to participate in this research study when you feel that you understand what is being asked of you, and you have had enough time to think about your decision.

Part 3: Purpose of the study

To assess the hematological abnormalities and their determinants among patients infected with *P. falciparum* and *P. vivax* at selected public health institutions in Bahir Dar city.

Part 4: Participant involvement and procedures

✓ **Participation is voluntary**

Participation in this study is entirely voluntary. If you decide to participate, you will be provided with a copy of this information sheet and asked to sign the consent form. You are free to withdraw at any time without having to give a reason. A decision to withdraw or not to take part, does not affect your rights in any way.

✓ **What will happen if you take part?**

The data collector will conduct an interview to collect socio-demographic and relevant clinical information from you. A drop of blood will be collected from your middle finger for smear preparation. If you are confirmed to have malaria, approximately 4 mL of venous blood and 2–3 g of stool will be collected aseptically for laboratory investigations. If the blood has already been collected once, no additional blood sample will be taken for the purpose of this study.

✓ **Duration of participation**

The overall process of participation in this study will take around 20-25 minutes, and no additional visit will be needed.

✓ **Participant responsibilities**

During the interview and blood sample collection, you are expected to provide honest responses and cooperate with the study protocols.

✓ **Possible benefits**

You will not receive any direct personal benefit such as money or materials. However, the findings of this study may contribute to the improvement of malaria diagnosis and support government management plans for malaria-related hematological abnormalities in the future.

✓ **Possible risks and discomforts**

Minor pain may occur at the blood collection site, but it is not expected to cause any major complications to your health. If any unexpected discomfort occurs, it will be managed accordingly by the attending health professionals.

✓ **If something goes wrong**

If any adverse events or side effects occur related to being a part of this study, they will be reported to the concerned body and the study participants will be assisted in accordance with the institution's care protocols.

✓ **Study completion and dissemination of results**

At the end of the study, participants will be informed about the dissemination of the research findings. Individual participants will not be listed by their name or specific address in any reports or publications. The results of this study will be published in scientific journals, and the participants will have access to the publications.

✓ **Contact for further information**

If you have any questions or concerns, they can contact the principal investigator.

Part 5: Confidentiality

All information collected from participants during the course of the research will be kept strictly confidential and will not be shared with anyone else. The information collected in this research study will be stored in a way that protects participant identities and will be maintained in accordance with legal requirements. The results of the study will be reported as group data, and individual participants will not be identified in any way.

Part 6: Summary

The research objectives and the contact details of the principal investigator such as name, email, and phone number will be provided to participants. Participation in this study is entirely voluntary, and they have the right not to take part in this study without negative consequences. Participants will be provided with a copy of this information sheet and a signed consent form.

Thank you for your time and contribution!!!

Annex 2: Information sheet (Amharic version)

ስለምርምሩ መረጃ

ክፍል 1: መግቢያ

የምርምር ጥናቱ ርዕስ

"በሰሜን ምዕራብ ኢትዮጵያ፤

ባሕር ዳር በሚገኙ በተመረጡ የህዝብ ጤና ተቋማት ውስጥ በጥላዝሞ ዲያግኖስቲክ ፓረም እና በጥላዝሞ ዲያግኖሽቲክ ስብስብ ያዙ ታካሚዎች ላይ የሚታዩ የደም ህዋሳት መዛባት፤ ንፅፅራዊ የክሮስ-ሴክሽናል ጥናት"

የሚልነው፤

አላማውም ለክሊኒካል ሂሳብ ለሚሰሩ ሌሎች እና አሙኖሂሳብ ለሚሰሩ ሌሎች የማስተርስ (ሁለተኛ) ዲግሪ ማሟያ መስፈርቶች አካል እንዲሆን ነው።

• የዋና ተመራማሪ

- ✓ ስም: አብርሃም ደሴአለሙ
- ✓ ተቋም: ባህር ዳር ዩኒቨርሲቲ፤ ህክምና እና ጤና ሳይንስ ኮሌጅ፤ ህክምና ላብራቶሪ ሳይንስ ትምህርት ቤት፤ ክሊኒካል ሂሳብ ለሚሰሩ ሌሎች እና አሙኖሂሳብ ለሚሰሩ ሌሎች ትምህርት ክፍል።
- ✓ ስልክ ቁጥር: 0988044564
- ✓ ኢሜይል: abrahamdessie16@gmail.com

ክፍል 2: በጥናቱ ለመሳተፍ የቀረበ ጥሪ

እርስዎ በዚህ የምርምር ጥናት እንዲሳተፉት ጋብዘዋል። ውሳኔዎን ወስንዎብዚ። ጥናቱ ለምን እንደሚካሄድ መረዳት አስፈላጊ ነው። ይህ የመረጃ ሉህ ስለ ጥናቱ ዓላማ፤ ስለ ሚያስከትለው ጉዳት እና ጥቅም ይገልጽ ሎታል። ግልጽ ያልሆነ ሎዎት ነገር ካለ፤ መረጃ ስብሰባ በውጤት ጨማሪ ማራራት ይሰጣል። በተጨማሪም መረጃውን ለማንበብ እና በውሳኔዎ ላይ ለማስብቁጥ ይረዳል። እርስዎ በዚህ የምርምር ጥናት ለመሳተፍ ከተስማሙ፤ የስምዎን ትቅጽ መፈረም ይኖርበታል።

ክፍል 3: የጥናቱ ዓላማ

የዚህ ጥናት ዓላማ በባህር ዳር ከተማ በሚገኙ በተመረጡ የህዝብ ጤና ተቋማት ውስጥ በጥላዝሞ ዲያግኖስቲክ ፓረም እና በጥላዝሞ ዲያግኖሽቲክ ስብስብ ያዙ ታካሚዎች ላይ የሚታዩ የደም ህዋሳት መዛባት (Hematological Abnormalities) ማወዳደር ነው።

ክፍል 4: የተሳታፊዎች ተሳትፎ እና ሂደቶች

- ✓ ተሳትፎ በፈቃደኝነት ላይ የተመሰረተ ነው

በዚህ ጥናት ላይ መሳተፍ ሙሉ በሙሉ በፈቃደኝነት የተመሰረተ ነው።

ያለ ምንምምክንያት በማንኛውም ጊዜ ከጥናቱ የማቋረጥ ነጻነት አለዎት።

ለማቋረጥ መወሰን ይምክሉ መሳተፍ የመታከም ሙሉ በሙሉ የምንምምንገድ አይነት ነው።

- ✓ በጥናቱ ቢሳተፉም ንይዘና ወናል

መረጃ ስብሰባ በወጣት የሰዓት ውስጥ በሀገራዊ እንዲሁም ተዛማጅ የሆኑ ክሊኒካዊ መረጃዎችን ለመስብሰብ ቃለመጠይቅ ያደርጉልዎታል።

በመቀጠልም ለስሚር (Smear) ዝግጅት ከመካከለኛ ጣት ዎክንድ ጠብታ ይመደብዎታል። በወባበሽታ መያዝ ዘመን 2-3g (የአተርፍሬ) የሚያከፍል ስግራ ይሰጣል። በተጨማሪም እንደ ሲቢሲ (CBC) ላለ የላቦራቶሪ ምርመራዎች 4 ሚሊሊትር ደም ከደም ስርወሰን ከሰጡት ነጻ በሆነ መንገድ ይወሰዳል። ደም አስቀድሞ እንደ ጊዜ ከተወሰደ፤

ለዚህ ጥናት ተብሎ ላተጨማሪ የደም ምርመራ ይወሰድበትም

- ✓ የተሳተፉ ፎቆይታ

በዚህ ጥናት የመሳተፍ አጠቃላይ ሂደት ከ 20-25 ደቂቃዎችን የሚወስድ ሲሆን፤ ምንም እንኳን ተጨማሪ ቀጠሮ አያስፈልግብዎትም።

✓ የተሳታፊውን ላፊነቶች

በቃለ-መጠይቁ እና በደምና ሙሉ አሰባሰብ ወቅት፤

ለሚጠየቁት ጥያቄዎች በታማኝነት በመመለስ እና የጥናቱን ፕሮቶኮል በመከተል ይኖርባቸዋል።

✓ ሊገኙ የሚችሉ ጥቅሞች

እንደ ገንዘብ ወይም ቁሳ ቁስ ያሉ ቀጥተኛ የግል ጥቅማጥቅሞችን አያገኙም።

ነገር ግን የዚህ ጥናት ውጤቶች የወጣበት ታምር መራን ለማሻሻል እና ወደፊት ከወጣ ጋር የተያያዙ የደም መዛባቶችን ለመቆጣጠር ለሚወጡ የመንግስት እቅዶች ድጋፍ ያበረክታሉ።

✓ ሊከሰቱ የሚችሉ ጉዳዮች

ደም በሚወሰድበት ቦታ ላይ መጠነኛ ህመም ሊሰማዎት ይችላል፤ ነገር ግን ይህ በእርሳዎ ጤና ላይ ከባድ ችግር አያስከትልም።

ማንኛውም ያልተጠበቀ ችግር ማጣት ካጋጠመዎት፤ በቦታው ባሉ የጤና ባለሙያዎች ተገቢው ህክምና ይደረግሎታል።

✓ ችግር በፈጠረ ወይም ያልተጠበቀ ነገር ከሰነድ

በዚህ ጥናት ውስጥ በመሳተፍ ምክንያት ማንኛውም ያልተጠበቀ የንጹሕ ሽንት ወይም አደጋ ቢከሰት፤

ለሚመለከተው አካል ሪፖርት ይደረጋል፤ እንዲሁም በተቋሙ የህክምና አሰጣጥ ደንብ (protocol) መሰረት ለእርስዎ አስፈላጊው እርዳታ ይደረግሎታል።

✓ የጥናቱ መጠናቀቅ እና የውጤት ሰርጭት

በጥናቱ መጠናቀቅ ላይ፤ ስለ ጥናቱ ውጤት ሰርጭት መረጃ ይሰጣል።

በማንኛውም ሪፖርት ወይም ህትመት ላይ የእርስዎ ስም እና አድራሻ በግል አይጠቀስም። የዚህ ጥናት ውጤቶች በሳይንሳዊ ጀርገኖች (መጽሔቶች) ላይ የሚታተሙ ሲሆን፤ እርስዎም እነዚህን ህትመቶች የማግኘት እድል ይኖርባቸዋል።

✓ ለተጨማሪ መረጃ

ማንኛውም ጥያቄ ወይም ቅሬታ ካልዎት፤ ዋና ተመራማሪውን ማግኘት ይችላሉ።

ክፍል 5: ሚስጥራዊነት

በጥናቱ ሂደት ከእርስዎ የሚሰበሰቡ ሁሉም መረጃዎች በጥብቅ ሚስጥራዊ ሆነው ይጠበቃሉ፤ ለሌላ ለማንም አካል አይሰጡም።

በዚህ ጥናት የተሰበሰቡ መረጃዎች የእርስዎን ማንነት በሚጠብቅ መልኩ የሚከማቹ ይሆናሉ።

የጥናቱ ውጤቶች በቡድን ደረጃ ሪፖርት የሚደረጉ ሲሆን፤ በምንም መንገድ የማንኛውንም ተሳታፊ መንግስት ሊያሳዩም።

ክፍል 6: ማጠቃለያ

በዚህ ጥናት ለመሳተፍ ፈቃደኛ ከሆኑ የጥናቱ ዓላማ እና የዋና ተመራማሪው አድራሻ (ስም፣ ኢሜይል እና ስልክ ቁጥር) ይሰጣሉ።

በዚህ ጥናት መሳተፍ ሙሉ በሙሉ ፈቃደኝነት ላይ የተመሰረተ ነው፤

እርስዎ ያለ ምንም አሉታዊ ተጽእኖ በዚህ ጥናት ያለ መሳተፍ መብት አዎት።

በተጨማሪም የዚህ የመረጃ ሉህ ቅጂ እና የተፈረመበት የስም ምንነት ቅጽ ከፈለጉ ይሰጣል።

ስለ ጊዜዎ እና ስለ ተዋጽኦዎ አመሰግናለሁ!!!!

Annex 3: Consent form (English version)

Dear Sir/Madam,

My name is -----, and I am anMSc student in Clinical Hematology and Immunohematology at Bahir Dar University. I am conducting a study entitled “Hematologic Abnormalities and Their Determinants among Patients Infectedwith *Plasmodium falciparum* and *Plasmodium vivax*in Bahir Dar City, Northwest Ethiopia.” The aim of this study is to assesshematological abnormalities and their determinantsamong malaria-infectedparticipants.

The interview willtake approximately 20–25 minutes. The information that you provide will bekept strictly confidential and will be usedfor research purposesonly. Your participation in this study is entirely voluntary. You have the right to refuse to answer any question or stop the interview at any time without any negative consequences.

There is no direct gainassociatedwith participating in this study. However, your honest responseswill contribute valuable information to improve patient care and understanding of malaria-related hematological changes.

If you agree to participate, please confirm your willingness below.

Do you agree to participate in this study?

Yes ()

No ()

Participant’sName -----Code: ----- Signature -----Date -----

Data collector’s Name----- Code: ----- Signature -----Date -----

Site/ Health Institution -----Code: -----

Thank you for your cooperation!!!

Annex 4: Consent form (Amharic version)

የስምምነት ቅጽ

ክቡር/ክቡርት፣

ስሜ _____ ይባላል። እኔ በባህር ዳርዩ ኒቨርሲ ቲየክሊ ኒካል ሂማቶ ሎጂ እና ኢሙኖሂማቶ ሎጂ የማስተርስ (የሁለተኛ ደረጃ) ተማሪ ነኝ። እየሰራሁት ያለሁት የምርምር ዕለት "በሰሜን ምዕራብ ኢትዮጵያ፣ ባሕር ዳር በሚገኙ በተመረጡ የህዝብ ጤና ተቋማት ውስጥ በፕላዝሞዲየም ፋልሲፓሪ ምእና በፕላዝሞዲየም ቫይረስ በተያዙ ታካሚዎች ላይ የሚታዩ የደም ህዋሳት መዛባት" የሚል ነው። የዚህ ጥናት ዓላማ በወቅታዊ ሚዛን ላይ ከፕላዝሞዲየም ፋልሲፓሪ ምእና በፕላዝሞዲየም ቫይረስ ጋር ተያይዘው የሚመጡትን የደም ህዋሳት መዛባት መወዳደር ነው።

ቃለ-መጠይቁ 20 እስከ 25 ደቂቃዎችን ይወስዳል።

የሚሰጡት መረጃ በጥቅሚያ ስፕራዊ ሆኖ የሚጠበቅ ሲሆን ለምርምር ዓላማ ብቻ ይውላል። በዚህ ጥናት ላይ የሚያደርጉት ተሳትፎ ሙሉ በሙሉ በፈቃደኝነት ላይ የተመሰረተ ነው። ማንኛውንም ጥያቄ ላለ መልስ ወይም በማንኛውም ሂደት ለምንም አይነት ቁጥጥር ያለ ቃለ-መጠይቁን የማቋረጥ መብት አለዎት። በዚህ ጥናት በመሳተፍ ዎ የሚገኙት ቀጥተኛ ጥቅም ወይም የሚደርስ በዎት ጉዳት የለም። ነገር ግን፣ የሚሰጡት ታማኝ ምላሽ የታካሚዎች ህክምናን ለማሻሻል እና ከወቅታዊ የተያያዙ የደም ለውጦችን ለመረዳት ጠቃሚ መረጃን ያበረክታል።

ለመሳተፍ ከተስማሙ፣ እባክዎ ፈቃደኝነትዎን ከዚህ በታች ያረጋግጡ፤

በዚህ ጥናት ለመሳተፍ ተስማምተዋል?

አዎ () አይ ()

የተሳታፊው ስም _____ ኮድ: _____ ፊርማ _____ ቀን _____

የመረጃ ሰብሳቢው ስም _____ ኮድ: _____ ፊርማ _____ ቀን _____

ቦታ/ የጤና ተቋም _____ ኮድ: _____

ስለ እውብርዎ እመሰግናለሁ!!!!

Annex 5: Questionnaire (English version)

Table 9: Socio-demographic and clinical information of study participants (English version)

Part I:- Socio-demographic information			
No	Questions	Response options	Remark
A01	Kebele name	-----	
A02	Health center name	1. Han 2. Shumabo 3. Shimbit 4. Addis Alem	
A03	Sex	1. Male 2. Female	
A04	Age (in years)	-----	
A05	Residence	1. Urban 2. Rural	
A06	What is your marital status?	1. Married 2. Single 3. Divorced 4. Widowed	
A07	What is your religion?	1. Orthodox 2. Muslim 3. Protestant 4. Other	
A08	What is your educational status?	1. Illiterate 3. Secondary (Grade 9 up to Grade 12) 2. Primary (Grade 1up to Grade 8) 4. College and above	
A09	What is your occupation?	1. Student 2. Government employee 3.Unemployed 4.Farmer 5.Others	
A10	How would you describe your household's economic status?	1. Low: poor housing (mud walls, earth floors, thatched roofs) no electricity, limited safe water, and pit latrine) 2. Medium: improved housing (brick walls, iron roofs), access to tap water and electricity, and flush toilet.) 3. High: good housing (concrete/stone walls, tiled floors), reliable water and electricity)	
Part II:- Medical history of participants			
B01	How many days have you been ill before coming for treatment?	1. ≤2 days 2. 3–5 days 3. 6–10 days 4. > 10 days	
B02	Have you had malaria before?	1. Yes 2. No	

Annex 6: Exclusion checklist (English version)

Table 10: Study participant eligibility and exclusion criteria checklist (English version)

No	Questions	Response options	Remark
Part I: Medical history of participants			
A01	Do you have any known chronic infection (e.g. HIV/AIDS, TB, kidney disease, or liver disease)?	1. Yes 2. No	If yes, exclude
A02	Do you have any known blood or hematological disorder (e.g. sickle cell disease, leukemia, or thalassemia)?	1. Yes 2. No	If yes, exclude
A03	Have you received blood transfusion in the past 3 months?	1. Yes 2. No	If yes, exclude
A04	Are you currently taking any medications that could affect your blood counts? (e.g. chemotherapy, heparin or immunosuppressants)	1. Yes 2. No	If yes, exclude
A05	Have you taken any malaria treatment in the last one month?	1. Yes 2. No	If yes, exclude
Part II: For female participants only (aged 15-45)			
B01	Are you currently pregnant?	1. Yes 2. No	If yes, exclude
B02	Are you unsure about your pregnancy status (missed period or suspect pregnancy)?	1. Yes 2. No	If yes, exclude

Annex 7: Questionnaire (Amharic Version)

Table 11: Socio-demographic and clinical information of study participants (Amharic version)

ክፍል አንድ፡ ማህበራዊ እና ስነ-ህዝባዊ መረጃ			
ተ.ቁ	ጥያቄዎች	የምላሽ አማራጮች	ምርመራ
A01	የቀበሌ ስም	-----	
A02	የጤና ተቋም ስም	1. ሀንጊ. ሹማአበ3. ሽምቢጥ 4. አዲስዓለም	
A03	ጾታ	1. ወንድ 2. ሴት	
A04	ዕድሜ (በዓመት)	-----	
A05	መኖሪያ	1. ከተማ 2. ገጠር	
A06	የጋብቻ ሁኔታ?	1. ያገባ/ች 2. ያላገባ/ች 3. የተፋታ/ች 4. የሞተበት/ባት	
A07	ሃይማኖት ያምንድንነው?	1. ኦርቶዶክስ 2. ሙስሊም 3. ፕሮቴስታንት 4. ሌላ ካለይ ገለጽ _____	
A08	የትምህርት ደረጃ?	1. ያልተማረ (ማንበብና መጻፍ የማይችል) 3. ሁለተኛ ደረጃ (ከ9ኛ-12ኛ ክፍል) 2. የመጀመሪያ ደረጃ (ከ1ኛ-8ኛ ክፍል) 4. ኮሌጅ እና ከዚያ በላይ	
A09	ሥራ ያምንድንነው?	1. ተማሪ 2. የመንግስት ሠራተኛ 3. ስራ አጥ 4. ገበሬ 5. ሌላ _____	
A10	የቤተሰብ ምን ዓይነት ሚዲያዎችን እንዴት ይገልጻሉ?	1. ዝቅተኛ፡ ደካማ የመኖሪያ ቤት (ከጭቃ የተሠራ ግድግዳ፣ የመሬት ወለል፣ በሳር የተከፈነ ጣራ)፣ የኤሌክትሪክ አገልግሎት የሌለው፣ ውስን የንጹህ መጠጥ ውሃ አቅርቦት እና የጉድጓድ ሽንት ቤት) 2. መካከለኛ፡ የተሻሻለ የመኖሪያ ቤት (የጡብ ግድግዳ፣ የቆርቆሮ ጣራ)፣ የቧንቧ ውሃ እና የኤሌክትሪክ አገልግሎት ተጠቃሚ፣ እንዲሁም የውሃ ፍሳሽ ሽንት ቤት 3. ከፍተኛ፡ ጥሩ የመኖሪያ ቤት (የኮንክሪት ወይም የድንጋይ ግድግዳ፣ የሴራሚክ ወለል፣ አስተማማኝ የውሃ እና የኤሌክትሪክ አቅርቦት)	
ክፍል ሁለት፡ የተሳታፊዎች የህክምና ታሪክ			
B01	እዚህ ከመምጣቶ በፊት በህመም ስንት ቀናት ቆዩ?	1. ≤2 ቀናት 2. 3–5 ቀናት 3. 6–10 ቀናት 4. > 10 ቀናት	
B02	ከዚህ በፊት ወባይ ዘዎት ያውቃል?	1. አዎ 2. አይ	

Annex 8: Exclusion checklist (Amharic version)

Table 12: Study participant eligibility and exclusion criteria checklist (Amharic version)

ተ.ቁ	ጥያቄዎች	የምላሽ አማራጮች	ምርመራ
ክፍል አንድ: የተሳታፊዎች የህክምና ታሪክ			
A01	የቆየ(ሥር) የሰደደኢ ንፌስ (በሽታ) አለብዎት? (ለምሳሌ: የኤች.አይ.ቪ/ኤድስ፣ የቲቢ፣ የጉበት ወይም የኩላሊት በሽታ)	1. አዎ 2. አይ	አዎከሆነ፣ ከጥናቱ አይካተቱ
A02	የታወቀ የደም ህመም ወይም መዛባት አለብዎት? (ለምሳሌ: የሲክል ሴል ህመም፣ ሉኪሚያ ወይም ታላሰሚያ)	1. አዎ 2. አይ	አዎከሆነ፣ ከጥናቱ አይካተቱ
A03	ባለፉት 3 ወራት ደምተሰጥቶታል?	1. አዎ 2. አይ	አዎከሆነ፣ ከጥናቱ አይካተቱ
A04	አሁን ላይ የደም ህዋስ ቆጠራ ንጊት ከሚቻል መድሃኒቶችን እየወሰዱ ነው? (ለምሳሌ: ኬሞቴራፒ፣ ሄፓሪን ወይም ኢሚኖሱፕሪሳንት)	1. አዎ 2. አይ	አዎከሆነ፣ ከጥናቱ አይካተቱ
A05	ባለፈው አንድ ወር ውስጥ ማንኛውንም የወሰደ መድሃኒት ወስደዋል?	1. አዎ አይ	2. አዎከሆነ፣ ከጥናቱ አይካተቱ
ክፍል ሁለት: ለሴት ተሳታፊዎች ብቻ (ዕድሜያቸው ከ15-45 ለሆኑ)			
B01	በአሁኑ ጊዜ ነፍስ ጦርነዎት?	1. አዎ 2. አይ	አዎከሆነ፣ አይካተቱ
B02	ስለ እርግዝና ሁኔታዎ እርግጠኛ አይደሉም? (የወር አበባ ቀርቶ በትክክል ይመስልዎታል እርግዝና የሚጠረጥሩ ከሆነ)?	1. አዎ 2. አይ	አዎከሆነ፣ አይካተቱ

Annex 9: Laboratory result registration form

Table 13: Laboratory result registration form of study participants

Part I: Microscopic examination results													
No	Questions							Response options				Remark	
A01	Peripheral blood smear result							1. <i>P. falciparum</i> 2. <i>P. vivax</i>					
A02	Are laboratory-confirmed intestinal parasites present?							1. Yes 2. No				If yes, exclude	
Part II: Complete blood count (CBC) results													
RBC count (×10 ¹² /L)	HBG (g/dL)	HCT (%)	RDW-CV(%)	MCV (fL)	MCH (pg)	MCHC(g/dL)	Total WBC (×10 ⁹ /L)	Neutrophil (×10 ⁹ /L)	Lymphocyte (×10 ⁹ /L)	Platelet count (×10 ⁹ /L)	MPV(fl)	PCT(%)	PDW(%)

Annex 10: Standard operating procedure for capillary blood sample collection

Purpose:

To provide uniform standards to be followed during capillary blood sample collection from participants to ensure safety, consistency, and reliability of samples for the purpose of this study.

Scope:

This SOP applies to the principal investigator, data collectors (trained laboratory professionals), and study participants involved in collecting, handling, labeling, transporting, and processing capillary blood samples for the purpose of this study.

Responsibilities:

- ✓ The principal investigator is responsible for supervising the adherence to this SOP and the aseptic collection of capillary blood samples.
- ✓ Trained laboratory professionals serving as data collectors are responsible for ensuring proper documentation, for conduct face to face interview, and for the aseptic collection of capillary blood samples.
- ✓ Study participants are responsible for following the instructions given by the principal investigator and data collectors.

Procedures:

1. Assemble all materials required for capillary blood sample collection (sterile lancets, 70% isopropyl alcohol, gloves, cotton, slides, biohazard container, and pencil).
2. Inform participants about the purpose of the capillary blood sample collection.
3. Confirm the study participant's identity and seat them comfortably.
4. Wash hands and wear clean disposable gloves.
5. Warm the selected puncture site (usually the 3rd or 4th fingertip for adults, and the heel for infants and neonates) and clean it with 70% isopropyl alcohol, and allow to air dry.
6. Make a 2–3mm deep puncture with a sterile lancet and wipe the first drop of blood with clean cotton.
7. Transfer the blood onto the slide and prepare blood smears.

- ✓ For the **thin film**, place a small drop of blood in the center of the slide and spread it at a 45° angle to form a feather-edge smear.
 - ✓ For the **thick film**, place a large drop of blood about 10mm from the thin film, away from the edge of the slide and the frosted end, and then spread it evenly in a circular manner.
8. Air-dry the slides and label them with participant's ID code.
 9. Stop the bleeding by applying gentle pressure to the puncture site preferably using sterile gauze pad.
 10. Thank the participant for their cooperation.

Infection prevention and waste disposal:

- Discard all disposable materials (needles, lancets, gloves, and contaminated gloves) immediately into a biohazard safety container after each procedure.
- Follow standard safety precautions to minimize the risk of infection.

Quality assurance (QA):

- Regularly supervise capillary blood sample collection and handling procedures to ensure adherence to this SOP.
- Document and quickly report any deviation from this SOP.

Safety precautions:

- Wear appropriate personal protective equipment (PPE) during blood sample collection.
- Provide appropriate first aid if participants experiencing discomfort or adverse reactions.

Annex 11: Standard operating procedure for venous blood sample collection

Purpose:

To provide standardized procedures to be followed during venous blood sample collection from participants to ensure safety, consistency, and reliability of samples for this study.

Scope:

This SOP applies to the principal investigator, data collectors (trained laboratory professionals), and the study participants involved in collection, handling, labeling, transporting, and processing of venous blood samples for this study.

Responsibility bodies:

- The principal investigator is responsible for supervising the adherence to this SOP and the aseptic collection of venous blood samples.
- Trained laboratory professionals serving as data collectors are responsible for ensuring proper documentation and for proper collection of venous blood samples.
- Study participants are responsible for following procedures stated in this SOP.

Procedures:

1. Assemble all materials required for venous blood sample collection, including a tourniquet, sterile needles, EDTA tube, 70% isopropyl alcohol, gloves, lab coat, and pencil.
2. Inform the study participants about the purpose of the venous blood sample collection.
3. Confirm the study participant's identity and seat them comfortably.
4. Apply the tourniquet 7–10 cm above the venipuncture site.
5. Clean the antecubital fossa using cotton moistened with 70% alcohol.
6. Support the participant's arm by holding it at the elbow or placing it on the table.
7. Insert the needle properly into the vein.
8. Advance the point of the needle at 0.5–1.0 cm into the subcutaneous tissue (at an angle of 45°, and then push forward at a lesser angle to pierce the vein wall).
9. Release the tourniquet at the moment blood starts to enter the syringe.

10. After drawing the required blood sample, remove the needle carefully and apply clean cotton to the puncture site.
11. Instruct the study participants to press the puncture site with cotton.
12. Finally, thank the participants for their cooperation.

Infection prevention and waste disposal:

- Follow standard precautions during venous blood collection.
- Wear appropriate PPE when drawing venous blood samples.
- Discard all disposable materials (needles, syringes, and contaminated gloves) immediately into a biohazard safety container after each procedure.

Quality assurance (QA):

- Regularly supervise about venous blood sample collection and proper handling procedures to ensure compliance with this SOP.
- Promptly document and report any deviation from this SOP.

Safety precautions:

- Wear appropriate PPE when drawing blood collection.
- Give appropriate first aid when participants experiencing discomfort or adverse reactions.

Annex 12: Standard operating procedure for Giemsa staining

Purpose:

To provide clear procedures for the preparation and storage of Giemsa stain used in this study.

Scope:

This SOP applies to the principal investigator and data collectors (trained laboratory professionals) during the Giemsa staining process for this study.

Responsibilities:

- The principal investigator is responsible for supervising adherence to this SOP and for preparing both working and stock Giemsa stains.
- Trained laboratory professionals serving as data collectors are responsible for ensuring proper documentation and for preparing both working and stock Giemsa stains.

Procedures:

1. Assemble all materials and reagents required for preparing both stock and working Giemsa stain (Giemsa powder, glycerol, absolute methanol, brown bottle, graduated cylinder, and balance).
2. Prepare a 500 mL of stock Giemsa stain solution by mixing 3.8g Giemsa powder with 250mL glycerol and 250 mL of absolute methanol.
3. Store the newly prepared stock Giemsa stain solution in a brown bottle for 2–3 days, and shake the solution 3–4 times daily.
4. Label the bottle with the name of the reagent and date of preparation, and store it at room temperature in a dark place.
5. Prepare 100mL of 10% working Giemsa stain by mixing 10mL of stock Giemsa solution with 90 mL of buffered water.
6. Allow the prepared blood smears to air-dry.
7. Fix the air-dried thin blood smear with absolute methanol for 10–20 seconds and allow it to air-dry.

8. Stain the smears with pre-prepared 10% working Giemsa stain for 10 minutes.
9. Wash the stained smears gently with tap water.
10. Clean the back of the slides using dry gauze.
11. Place the slides film side down in a drying rack to drain.

Quality assurance (QA):

- Use known positive and negative control slides with each new batch of working Giemsa stain.
- Store the prepared stock Giemsa stain at room temperature and protected from direct sunlight.

Safety precautions:

- Clearly label Giemsa stain as a flammable substance and follow standard precautions strictly to reduce associated risks.
- Dispose of leftover Giemsa stain in accordance with the local laboratory waste management system.

Annex 13: Standard operating procedures for malaria microscopic diagnosis

Purpose:

To provide procedures for the accurate detection and species identification of malaria parasites in thin and thick blood films using a light microscope.

Scope:

This SOP applies to all personnel performing malaria microscopy, including the data collectors (trained laboratory professionals) and the principal investigator for this study.

Responsibilities:

- The principal investigator is responsible for ensuring the implementation of and compliance with this SOP and for performing malaria microscopy.
- Trained laboratory professionals serving as data collectors are responsible for strictly following standard microscopy procedures.

Procedures:

1. Apply a drop of immersion oil on the dried, stained blood smear.
2. Place the slide on the mechanical stage of the microscope.
3. Scan the entire smear at low magnification.
4. Examine the smear using the 100× oil immersion objective.
5. Check for the presence of parasites on the thick smear and identify species on the thin smear.
6. Remove the slide from the mechanical stage of the microscope.

Quality assurance (QA):

- Check the microscope to ensure it is free of dust and properly aligned.
- Check all lenses as well as the slides to ensure they are free of scratches.

Safety precautions:

- Always cover the microscope when not in use.
- Store the microscope in a clean, dry, stable environment away from direct sunlight.

Annex 14: Standard operating procedure for hematological analyzers

Purpose:

To ensure the proper functioning of the hematological analyzers (Mindray BC-20S, Zybio Z3, and HTI MicroCC-20Plus) and provide standardized procedures for processing participants samples during the study.

Scope:

This SOP applies to all procedures performed using the Mindray BC-20S, Zybio Z3, and HTI MicroCC-20Plus hematology analyzers during this study.

Responsibilities:

- The principal investigator is responsible for ensuring the effective implementation of this SOP and for performing the procedure.
- Trained laboratory professionals serving as data collectors are responsible to follow this SOP strictly and correctly performing all procedures.

Sample and container type:

- Sample type: Whole blood specimen
- Amount required: 4 mL (to ensure the proper blood to anticoagulant ratio).
- Transport and Storage: Ambient temperature.
- Stability: 6–8 hours at room temperature.
- Container type: K3 EDTA anticoagulated (purple-top) tube

Procedures:

1. Collect 4 mL venous blood sample into EDTA tubes following standard venipuncture procedures.
2. Label each tube with a unique study identification number.
3. Mix the samples gently by inversion before analysis.
4. Set up the hematological analyzers according to routine laboratory operating procedures.
5. Enter the necessary information into the analyzer.
6. Use the “whole blood mode” for participant with sufficient sample volume.

7. Aspirate the samples according to analyzers operating procedures.
8. Recoded and interpret the results numerically as displayed on the screen.
9. Check each hematological parameter for the presence of hematologic abnormalities (anemia, thrombocytopenia, and leucopenia) according to the operational definitions.

Quality assurance (QA):

- Perform daily internal quality control (IQC) by using routine QC procedures according to the manufacturer's instructions for each hematology analyzer.
- Review any abnormal instrument flags and repeat the analysis when necessary.

Safety measures:

- Follow manufacturer's safety precautions.
- Use cleaning and lysing solutions carefully manufacturer's instructions.
- Maintain the power supplies carefully.
- Ensure both the data collectors and the principal investigator trained on the operation of the machine.

Annex 15: Standard operating procedure for stool microscopy

Purpose:

To detect the diagnostic stages of intestinal parasites in stool samples using wet mount preparation technique.

Scope:

This SOP applies to the principal investigator and the data collectors (trained laboratory personnel) during stool microscopy procedures conducted for this study, as well as to study participants during stool sample collection.

Responsibilities:

- The principal investigator is responsible for supervising adherence to this SOP and performing stool microscopy.
- Trained laboratory personnel serving as data collectors are responsible for ensuring proper documentation and for performing stool microscopy.
- The study participants are responsible for collecting stool samples as instructed and strictly following this SOP.

Procedures:

1. Assemble all materials and reagents required for stool wet mount (clean slide, cover slips, saline, microscope, applicator stick, gloves, lab coat, and biohazard container).
2. Instruct the study participants to collect a pea-sized stool in clean and labeled container.
3. Add a drop of saline solution onto the labeled slide.
4. Add approximately 2–3g of stool to the saline drop.
5. Mix the stool and the saline drop with applicator stick until a uniform suspension is formed.
6. Place a cover slip over the suspension.
7. Examine the slides under a light microscope, starting with low power objectives (10×), followed by high power objective (40×) for detailed observation.
8. Discard slides, cover slips, and remnant stool samples into biohazard container.
9. Finally, disinfect the microscope and working area.

Quality assurance (QA):

- Use fresh stool samples to ensure the viability of some parasites.
- Use appropriate concentration of normal saline (0.85%).
- Use standard examination pattern (zig-zag) for consistent slide reading.
- Have another laboratory personnel re-check the results for verification.

Safety measures:

- Wear the appropriate PPE during processing stool sample.
- Disinfect the working area before and after each work with bleach.
- Properly discard contaminated slides, left over stool samples, and applicators into the biohazard container immediately after use.

13. Declaration

This is to certify that the thesis entitled, "Hematologic Abnormalities and Their Determinants among Patients Infected with *Plasmodium falciparum* and *Plasmodium vivax* in Bahir Dar, Northwest Ethiopia: A Cross-Sectional Study," submitted in partial fulfillment of the requirements for the Master's degree in Clinical Hematology and Immunohematology at Bahir Dar University, is my original work and has never been submitted to this or any other institution to obtain any other degree or certificate. The assistance and support I received during the course of this study have been duly acknowledged.

Principal investigator

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13. Conflict of interest

I hereby declare that the research entitled, “Hematologic Abnormalities and Their Determinants among Patients Infected with *Plasmodium falciparum* and *Plasmodium vivax* in Bahir Dar, Northwest Ethiopia: A Cross-Sectional Study,” was conducted with no commercial or financial conflicts of interest.

Name:-

Signature:-

Date:-