ORIGINAL RESEARCH

Hematological Abnormalities Among Malaria Infected Adult Patients in Association with ABO Blood Groups at Jinella Health Center, Harar, Eastern Ethiopia

Haftu Asmerom (ID), Kabtamu Gemechu (ID), Beza Sileshi, Mesay Arkew (ID)

School of Medical Laboratory Sciences, College of Health and Medical Sciences, Haramaya University, Harar, Ethiopia

Correspondence: Haftu Asmerom, School of Medical Laboratory Sciences, College of Health and Medical Sciences, Haramaya University, P.O. Box: 235, Harar, Ethiopia, Tel +251910089878, Email wawhaftu12@gmail.com

Background: Hematological abnormalities are a common complication of malaria infection. However, there is a paucity of evidence regarding it among malaria-infected adult patients in association with the ABO blood group in Ethiopia, particularly in the Harari Region. Therefore, this study aimed to assess the hematological abnormalities among malaria-infected adult patients in association with ABO blood groups at Jinella Health Center, Harar, Eastern Ethiopia.

Methods: An institutional-based cross-sectional study was conducted from July 10, 2022, to January 10, 2023. Four milliliters of venous blood were collected from each study participant. Drops of blood were used for blood film preparation. ABO blood group was determined by agglutination test using monoclonal anti-sera (Agape Diagnostics Ltd., India). A complete blood count was done using the DxH 800 (Beckman Coulter, Inc, Miami, FL) hematology analyzer. The data were analyzed using SPSS version 26. Bivariable and multivariable logistic regression models were fitted. The level of significance was declared at a p-value of <0.05.

Results: The study revealed that 47.2% (95% CI: 41.0 53.6) of the participants were anemic. Being female (AOR = 3.18, 95% CI = 1.67, 6.04), having the A blood group (AOR = 2.75, CI = 1.20, 6.31), and being infected with *P. falciparum* (AOR = 2.64, CI = 1.26, 5.53) were all significantly associated with malaria anemia. The overall prevalence of thrombocytopenia was also 67.7% (95% CI: 61.7–73.4%). It was significantly associated with *P. falciparum* infection (AOR = 8.03, CI = 3.53, 18.25) and high parasitemia levels (AOR = 4.40, CI = 1.57, 12.32).

Conclusion: Patients with malaria who belonged to the "A" blood group in the study area had anemia as a serious health problem. Hence, frequently checking for anemia in patients with malaria who have blood group "A" can help with early detection and better management of anemia.

Keywords: malaria, hematological abnormalities, blood groups, Harar, Ethiopia

Background

The two *Plasmodium species, Plasmodium falciparum (P. falciparum)* and *Plasmodium vivax (P. vivax)* are the principal causes of malaria and pose the greatest risks to the world's population.[1] The most frequent side effects of malaria infection are hematological abnormalities, which affect red blood cells (RBC), white blood cells (WBC), and platelets (PLT).[2,3]

Hematological abnormalities are caused by *P. falciparum*-encoded polypeptides (*RIFINs*), sub-telomeric variants (*STEVOR*), and *P. falciparum* erythrocyte membrane protein 1 (*PfEMP1*) to ensure antigenic variation and evasion of host immunity and mediate the binding of parasite-infected RBC to the vascular endothelium (cytoadherence), to RBC (rosetting), to WBC, and sequestration of cells.[4–6] The expression of *STEVOR* on the RBC leads to *PfEMP1*-independent binding of infected RBCs to uninfected RBCs (rosette formation), while antibodies targeting *STEVOR* in the merozoite can effectively inhibit invasion.[7]

The A, B, and H carbohydrate antigens on the surface of RBC make up the ABO blood group system, which is the most significant one in clinical practice when it comes to the relationship between malaria infection.[8] It is one of the hereditary factors that may affect a person's vulnerability to the clinical effects of malaria.[5,8–10] Anemia, thrombocytopenia, leukopenia, and leukocytosis are hematological abnormalities that have been associated with malaria infection and are thought to be its distinguishing feature.[11]

Cytoadherence and rosetting contribute to the pathophysiology of malaria by obstructing blood flow, resulting in a shortage of oxygen in tissues, excessive lactate formation, and a decline in the pH of blood and tissues, which can result in severe anemia.[5] It is characterized by hemoglobin (Hgb) levels that are below 12 g/dl for women and 13 g/dl for males.[12]

Another very common malarial consequence is thrombocytopenia, which is characterized by low PLT levels ($<150 \times 10^3$ cells/μL).[13] Leukopenia and leukocytosis are also seen throughout the world, notably in Africa.[14,15] A study carried out in Ethiopia also revealed leucopenia and leukocytosis among malaria-infected patients.[16] The ABO blood group is one of the genetic factors linked to human susceptibility to severe malaria infection.[5] Some researchers have looked at the molecular nature of the interaction between the ABO blood group and *P. falciparum* via the binding of parasitized RBC to RBC.[5,17] Yet, and this is crucial, the ABO blood group antigens are equally expressed on the vascular endothelium and serum proteins, indicating that they may be responsible for the development of severe malaria.[18]

According to several studies, people with blood groups A and B are more likely to develop severe malaria than people with blood group O[19,20] and have a higher frequency of anemia.[21] On the other hand, a few studies revealed that people with the O blood group had the highest proportion of malaria infections, followed by those with the A blood group.[19,22] Hematological abnormalities have been observed in malaria patients. Nevertheless, there is not much information available regarding the prevalence of anemia, thrombocytopenia, leukopenia, and leukocytosis in association with the ABO blood groups. Therefore, this study aimed to assess the hematological abnormalities among malaria-infected adult patients in association with ABO blood groups at Jinella Health Center, Harar, Eastern Ethiopia.

Materials and Methods
Study Design, Area, and Period
A cross-sectional study was conducted in the Harar region, which is one of the ten regional states of the Federal Democratic Republic of Ethiopia, with Harar as the capital city. This region is found 526 kilometers east of Addis Ababa, the capital city of Ethiopia. According to the Harari Regional Health Bureau's 2018 annual report, the region consists of four hospitals (two of which are public and private hospitals), and ten health centers. Of those ten health centers, the study was carried out at Jinella Health Center from July 10, 2022, to January 10, 2023.

Study Participants
The study population consisted of all adult patients (aged between 18 and 65 years old) who had been confirmed to have malaria and gave their consent to take part in the study. Contrarily, individuals who were positive for infectious diseases (Human Immunodeficiency Virus (HIV), Hepatitis B Surface Antigen (HBsAg), and Hepatitis C Virus (HCV)), patients who were pregnant, patients who had intestinal parasites, and patients with history of chronic disease (hypertension, heart disease, and diabetes mellitus) obtained through clinical records or patient cards were excluded from the study.

Sample Size Determination and Sampling Technique
The sample size for the prevalence of thrombocytopenia among malaria-infected patients was determined using single population proportion formula considering the following assumptions: 95% confidence level, 5% margin of error, and proportion of thrombocytopenia among malaria-infected patients conducted in Ethiopia (84%).[23] This provided us with a sample size of 206. The sample size for the hematology abnormality in association with ABO blood groups was calculated in Open Epi Info 7.2.5 statistical software assuming the following assumptions: 95% confidence level, 80% power, equal unexposed to the exposed ratio (1:1), the proportion of anemia among malaria patients of A blood group (41.6%) and B blood group (22.9%).[21] This provided a sample size of 231. The sample size calculated for the proportion

of malaria anemia among blood groups (231) was used for this study as it was greater than the calculated sample size for thrombocytopenia among malaria-infected patients. After the addition of 10% of non-responders, a total of 254 study participants were recruited using a convenience sampling technique.

Data Collection Procedure

After being written in English, the questionnaire was translated into two regional dialects (Amharic and Afan Oromo), and then back into English. Before data collection, collectors selected patients who qualified for the inclusion criteria and obtained informed consent. Socio-demographic information, clinical records, and medical history of patients were collected by administering the questionnaire under the supervision of the principal investigator.

Laboratory Sample Collection and Malaria Diagnosis

Four mL of venous blood was drawn from each patient and put into ethylene diamine tetraacetic acid (EDTA) tubes using a syringe and needle. For microscopic analysis, 1 drop (about 30 µL) and 2 drops (about 60 µL) of blood from each EDTA test tube were used to prepare thin and thick blood films on labeled slides, respectively. Methanol was used to fix the thin film for 10 seconds. Later, both films were stained with 10% Giemsa for 10 minutes, then carefully washed and air-dried. The slides were examined under a light microscope with an oil immersion (x100) objective.[24] The parasitemia level was calculated on a thick blood film using the following formula; $\text{Parasites}/\mu\text{L blood} = \frac{\text{Number of parasites counted X 8000 WBC}/\mu\text{L}}{\text{Number of WBC counted}}$[25] and it was defined as high parasitemia (>10 parasites per field), moderate parasitemia (1–10 parasites per field), and low parasitemia (1–100 parasites per 100 fields).[12]

Blood Group Determination

ABO blood groups were typed using commercially available monoclonal antisera (Agape Diagnostics Ltd., India). Two drops of whole blood from an EDTA test tube were placed in each of three grease-free, clean glass slides on which the same proportion (two drops) of antisera for blood groups A, B, and Rh was applied. The blood and the antisera were mixed with an applicator stick. The slide was then tilted to detect agglutination, and the result was recorded accordingly as blood groups A+, B+, AB+, and O+, or A-, B-, AB-, and O-.[26]

Determination of Hgb Concentration, Total WBC, and PLT Counts

The remaining blood sample in the EDTA test tube was used to determine Hgb concentration, RBC, total WBC, and PLT counts using an automated hematology analyzer UniCel DxH 800 Coulter (Beckman Coulter, Inc, Miami, FL), following the protocol of the manufacturer. Major blood cells such as Hgb, RBC, total WBC count, and PLT were recorded. To define anemia in the patients, hemoglobin values of <12 g/dl for women and <13g/dl for men were used. Anemia was further classified as mild (Hgb 10.0–10.9 g/dl), moderate (Hgb 7–9.9 g/dl), and severe (Hgb <7g/dl).[27,28] A WBC count of >10,000 cells/µL was classified as leukocytosis, while leucopenia was defined as a WBC count <4000 cells/µL.[29] A PLT count <150, 000 cells/µL was used to define thrombocytopenia. It was further classified as mild (PLT between 100,000 and 150,000 cells/µL), moderate (PLT between 50,000 and 100,000 cells/µL), severe (PLT <50, 000 cells/µL).[30,31]

Statistical Analysis of the Data

Following data collection, the data were edited and cleaned, and each questionnaire's completion and coding were verified. Data were entered into Epi-Data version 4.4.2.1, and all data were edited, organized, coded, exported, and analyzed using Statistical Package for Social Sciences (SPSS) version 26 statistical software. Tables, percentages, and frequencies were used to describe categorical variables. The normal distribution of continuous data was examined using the Kolmogorov–Smirnov test. Bivariable logistic regression analysis was used, and a Crude Odds Ratio (COR) with a 95% confidence interval (CI) was computed to assess the association between each independent variable and the outcome variables. Variables with a p-value of < 0.25 were considered candidates for multivariable logistic regression analysis. Model goodness-of-fit was checked by the Hosmer and Lemeshow test, and the final model was well-fitted with

the included independent variables (P value = 0.82). The final model was performed to control the confounding variables and identify the associated factors by estimating the Adjusted Odds Ratio (AOR) with a 95% CI. Statistical significance was declared at a p-value of < 0.05.

Data Quality Control

The questionnaire was translated into the local dialects (Afan Oromo and Amharic) for data collection and then translated back into English. A week before the actual data collection, 5% of the questionnaires were pre-tested among adult patients having malaria at the Babile Health Center, which is about 24.9 kilometers from the study area. The completeness of each questionnaire was checked by the investigators daily. All laboratory activities were performed with strict adherence to standard operating procedures (SOP). The quality and expiration dates of the methanol used for thin blood fixation and Giemsa stain were checked. Giemsa was used to stain positive malaria slides to ensure the product's quality. Before deeming the slide negative, up to 100 high-power fields were viewed. An expert laboratory technologist reviewed any discrepancies in the blood film results between the two laboratory professionals. Strict adherence to the automated hematology instructions was observed. Quality checks were carried out daily using low, normal, and high control levels to track an instrument's performance over time before beginning sample analysis.

Ethical Consideration

The ethical approval was received from the Institutional Health Research Ethics Review Committee (IHRERC), College of Health and Medical Sciences, Haramaya University. Informed, voluntary, written, and signed consent was obtained before the initiation of the study from each participant and the health center head. Both the measurements and the interviews took place in private settings in different rooms. All possible identifiers were excluded from the questionnaires to protect participant confidentiality. The study was carried out in accordance with the Declaration of Helsinki.

Results

Socio-Demographic Characteristics

Of the total of 254 malaria-infected patients who took part in the study, 139 (54.7%) were male. The highest 86 (33.9%) number of participants was found to be within the age range of 18–27 years, while the least number was found to be within the age range of 58–65 years. One hundred thirty-six (53.5%) of the respondents were married, and 7 (2.8%) were widowed. The majority, 144 (56.7%) were living in rural areas, and 93 (36.6%) were able to write and read. Regarding occupational status, the highest 70 (27.5%) number of the study participants were farmers (Table 1).

Association of ABO Blood Group with Malaria Infection

The ABO blood group of all malaria patients was also examined. Accordingly, the highest prevalence of malaria infection was observed among subjects with blood groups A and B, with a prevalence of 91 (35.8%) and 77 (30.3%), respectively. While individuals with blood group AB (34 (13.4%)) were the least affected. Predominantly, 64 (70.3%) A blood group, 21 (61.8%) AB blood group, and 25 (48.1%) O blood group patients were infected with *P. falciparum* whereas the highest 36 (46.8%) of B blood group malaria patients were infected with *P. vivax*. Eleven (12.1%) A blood group patients and 5 (14.7%) AB blood group patients were infected with mixed infections. Overall, there was a statistically significant association between ABO blood groups and malaria severity (χ^2 = 19.575, p = 0.003). Regarding the Rh factor, the majority of 226 (89.0%) malaria patients were Rh-positive. One hundred twenty-seven (88.2%), 71 (88.8%), and 28 (93.3%) malaria-infected with *P. falciparum, P. vivax*, and mixed infection were Rh-positive, respectively. But there was no statistically significant association between the Rh factor and malaria severity (χ^2 = 0.675, p = 0.71). (p = 0.71) (Table 2).

Association of ABO Blood Groups with Anemia

The mean hemoglobin level of the study participants was 12.1g/dl ± 2.8 and ranged from 5.7 to 17.4 g/dl. The overall prevalence of anemia among malaria-infected adult patients was 47.2% (95% CI: 41.0–53.6). Out of this prevalence, 53

Table 1 Socio-Demographic Characteristics of the Study Participants at Jinella Health Center, Harar, Eastern Ethiopia from July 10, 2022-January 10, 2023 (N = 254)

Variables	Category	Malaria Patients N (%)
Age in years	18–27	86 (33.9)
	28–37	70 (27.6)
	38–47	41 (16.1)
	48–57	38 (15.0)
	58–65	19 (7.5)
Sex	Male	139 (54.7)
	Female	115 (45.3)
Marital Status	Single	97 (38.2)
	Married	136 (53.5)
	Divorced	14 (5.5)
	Widowed	7 (2.8)
Residence	Urban	110 (43.3)
	Rural	144 (56.7)
Educational Status	Unable to read and write	73 (28.7)
	Able to read and write	93 (36.6)
	Preparatory school	46 (18.1)
	College and above	42 (16.5)
Occupational Status	Governmental	51 (20.1)
	Merchant	46 (18.1)
	Student	42(16.5)
	Farmer	70(27.5)
	Housewife	38 (15.0)
	Other	7 (2.8)

Abbreviation: N, Number.

Table 2 Cross-Tabulation of Chi-Square Analysis of ABO Blood Groups Among Malaria-Infected Adult Patients at Jinella Health Center, Harar, Eastern Ethiopia from July 10, 2022-January 10, 2023 (N=254)

Blood Group System		Number of Examined	Infection Type			χ^2, P-value
			P. falciparum	*P. vivax*	Mixed	
Blood group	A	91	64(70.3)	16(17.6)	11(12.1)	χ^2 =19.575
	B	77	34 (44.2)	36(46.8)	7(9.1)	P=0.003
	AB	34	21(61.8)	8(23.5)	5(14.7)	
	O	52	25(48.1)	20(38.5)	7(13.5)	
Rh Type	Rh (+)	226	127(56.2)	71(31.4)	28(12.4)	χ^2 =0.675
	Rh (-)	28	17(60.7)	9(32.1)	2(7.1)	P=0.714

Abbreviations: N, Number; *P. falciparum, Plasmodium falciparum*; *P. vivax, Plasmodium vivax*; Mixed, an infection of *P. falciparum* and *P. vivax*; Rh (+), Rh positive; Rh (-), Rh negative; χ^2, Chi square.

(20.9%), 60 (23.6%), and 7 (2.8%) had mild, moderate, and severe anemia, respectively. Blood group A individuals (51.7%) had the highest prevalence of anemia, followed by blood group B individuals (22.5%). The lowest prevalence of anemia was 7.5% among blood group AB individuals. The most severe anemia was detected in individuals with blood group A (5 (71.4%)). Twenty-eight (46.7%) and 16 (26.7%) of the A and B blood groups, respectively, showed the highest levels of moderate anemia. The highest mild anemia appeared among 29 (54.7%) of blood group A individuals,

Table 3 Cross-Tabulation of Chi-Square Analysis of ABO Blood Groups with Anemia Among Study Participants at Jinella Health Center, Harar, Eastern Ethiopia from July 10, 2022-January 10, 2023 (N =120)

Blood Group System		Anemia Classification				χ^2, P-value
		Mild	Moderate	Severe	Non-Anemic	
Blood Group	A	29(54.7)	28(46.7)	5(71.4)	29(21.6)	χ^2 =30.262
	B	11(20.8)	16(26.7)	0(00)	50(37.5)	P < 0.001
	AB	3(5.7)	5(8.3)	1(14.3)	25(18.7)	
	O	10(18.9)	11(18.3)	1(14.3)	30(22.4)	
Rh Type	Rh (+)	47(88.7)	54(90.0)	5(71.4)	120(89.6)	χ^2 =2.312
	Rh (-)	6(11.3)	6(10.0)	2(28.6)	14(10.4)	P =0.510

Abbreviations: N, Number; Rh (+), Rh positive; Rh (-), Rh negative; χ^2, Chi square.

and the lowest was among 3 (5.7%) of blood group AB individuals. There was a statistically significant association between the ABO blood group and the anemia status of the study participants (p < 0.001). The majority of mild, moderate, and severe anemias were Rh (+), with proportions of 47 (88.7%), 54 (90.0%), and 5 (71.4%), respectively (Table 3).

Association of ABO Blood Groups with Thrombocytopenia

The mean platelet value of the study participants was 140×10^3 cells/μL± 84.3 and ranged from 39 to 405×10^3 cells/μL. The overall prevalence of thrombocytopenia was 67.7% (95% CI: 61.6–73.4). The highest prevalence of thrombocytopenia was detected among individuals with blood group A (67 (39.0%)), followed by blood group B (47 (27.3%)). The lowest prevalence of thrombocytopenia was 24 (14.0%) in blood group AB individuals. Out of the total participants, 84 (33.1%), 60 (23.6%), and 28 (11.0%) had mild, moderate, and severe thrombocytopenia, respectively. The most severe thrombocytopenia was detected in individuals with blood group B (11 (39.6%)). However, the highest prevalence of moderate and mild thrombocytopenia was found among blood group A participants, with a prevalence of 22 (36.7%) and 36 (42.9%), respectively. There was no statistically significant association between the ABO blood group and thrombocytopenia in the study participants (p = 0.319). The majority of mild, moderate, and severe thrombocytopenia were Rh (+), with proportions of 71 (84.5%), 55 (91.7%), and 27 (96.4%), respectively (Table 4).

Association of ABO Blood Group with WBC Alteration

The mean WBC value of the study participants was 6.1×10^3 cells/μL± 3.1 and ranged from 2.2 to 14.1×10^3 cells/μL. Of the total number of study participants, 81 (31.9%) of them had leukopenia, and 33 (13.0%) of them also had leukocytosis. The highest number of leukopenia was observed among participants with blood group A (32 (39.5%)), followed by blood group O (21 (25.9%)). However, the lowest prevalence of leukopenia was 11 (13.6%) among blood

Table 4 Cross-Tabulation of Chi-Square Analysis of ABO Blood Groups with Thrombocytopenia Among Malaria-Infected Adult Patients at Jinella Health Center, Harar, Eastern Ethiopia from July 10, 2022-January 10, 2023 (N =172)

Blood Group System		Thrombocytopenia Classification				χ^2, P-value
		Mild	Moderate	Severe	No-Thrombocytopenia	
Blood Group	A	36(42.9)	22(36.7)	9(32.1)	24(29.3)	χ^2 =10.396
	B	21(25.0)	15(25.0)	11(39.3)	30(36.6)	P= 0.319
	AB	9(10.9)	13(21.7)	2(7.1)	10(12.2)	
	O	18(21.4)	10(16.7)	6(21.4)	18(22.0)	
Rh Type	Rh (+)	71(84.5)	55(91.7)	27(96.4)	73(89.0)	χ^2 =3.726
	Rh (-)	13(15.5)	5(8.3)	1(3.6)	9(11.0)	P=0.293

Abbreviations: N, Number; Rh (+), Rh positive; Rh (-), Rh negative; χ^2, Chi square.

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Table 5 Cross-Tabulation of Chi-Square Analysis of ABO Blood Groups with WBC Alterations Among Malaria-Infected Adult Patients at Jinella Health Center, Harar, Eastern Ethiopia from July 10, 2022-January 10, 2023 (N =114)

Blood Group System		White Blood Cell Alterations			χ^2, P-value
		Leukopenia	Leukocytosis	Normal	
ABO Blood Group	A	32(39.5)	9(27.3)	50(35.7)	χ^2 =7.934
	B	17(21.0)	11(33.3)	49(35.0)	P= 0.243
	AB	11(13.6)	4(12.1)	19(13.6)	
	O	21(25.9)	9(27.3)	22(15.7)	
Rh Type	Rh (+)	72(88.9)	29(87.9)	125(89.3)	χ^2 =0.055
	Rh (-)	9(11.1%)	4(12.1)	15(10.7)	P=0.973

Abbreviations: N, Number; Rh (+), Rh positive; Rh (-), Rh negative; χ^2, Chi square.

group AB individuals. Seventy-two (88.9%) who had leukopenia were RH (+). Regarding leukocytosis, the highest number of leukocytosis was detected in blood group B (11 (33.3%)). However, the least leukocytosis appeared among blood group AB (4 (12.1%)) participants. Twenty-nine (87.9%) study participants who had leukocytosis were RH (+). There was no statistically significant association between the ABO blood group, Rh type, and WBC alterations (P = 0.243) (Table 5).

Factors Associated with Anemia

Bivariable and multivariable binary logistic regression analyses were performed to determine parameters associated with the anemia status of adult malaria patients. In the bi-variable binary logistic regression analysis; sex, marital status, educational status, parasitemia level, *Plasmodium species*, and blood group were significant at a p-value of <0.25 and considered candidates for multivariable logistic regression analysis. In multivariable logistic regression, sex, *Plasmodium species*, and blood group were found to have a significant association with anemia at a p-value of <0.05. The odds of being anemic among female malaria-infected patients were 3.2 times more likely (AOR = 3.18, 95% CI = 1.67, 6.04) than their male counterparts. Malaria patients infected with *P. falciparum* were 2.64 times more likely (AOR = 2.64, CI = 1.259, 5.53) to develop anemia than those infected with *P. vivax*. Blood group A malaria-infected patients were 2.75 times more likely (AOR = 2.75, CI = 1.20, 6.31) to develop anemia than blood group O patients (Table 6).

Factors Associated with Thrombocytopenia

Both bi-variable and multivariable binary logistic regression analyses were conducted to identify factors associated with thrombocytopenia in malaria-infected adult patients. In the bi-variable binary logistic regression analysis; sex, educational status, occupational status, parasitemia level, and *Plasmodium species* were significantly associated at a p-value of <0.25 and considered candidates for multivariable logistic regression analysis. In multivariable logistic regression, *Plasmodium species* and parasitemia level were found to have a significant association with thrombocytopenia at a p-value of <0.05. Malaria patients infected with *P. falciparum* were 8.03 times more likely (AOR = 8.03, CI = 3.53, 18.25) to develop thrombocytopenia than those infected with *P. vivax*. Malaria-infected patients with a high parasitemia level were 4.40 times more likely (AOR = 4.40, CI = 1.57, 12.32) to develop thrombocytopenia than malaria-infected patients with a low parasitemia level (Table 7).

Discussion

Hematological abnormalities are one of the most common complications of malaria infection, along with malarial anemia, thrombocytopenia, and leukocytosis or leucopenia.[12] The findings of this study assessed the hematological abnormalities among malaria-infected adult patients in association with ABO blood groups at Jinella Health Center, Harar, Eastern Ethiopia. Accordingly, the hemoglobin test result showed that 47.2% of study participants were anemic. Being female, having an "A" blood group, and being infected with *P. falciparum* were identified as associated factors of anemia among malaria-infected patients in association with ABO blood groups. On the other hand, A PLT count <150,

Table 6 Logistic Regression Analysis of Associated Factors with Anemia Among Malaria-Infected Adult Patients at Jinella Health Center, Harar, Eastern Ethiopia from July 10, 2022-January 10, 2023 (N=254)

Variables	Category	Anemia Status		95% CI	
		Yes N (%)	No N (%)	COR	AOR
Age in years	18–27	43(50.0)	43(50)	1	1
	28–37	35(50.0)	35(50.0)	1.000(0.532, 1.880)	1.164(0.500, 2.713)
	38–47	18(43.9)	23(56.1)	0.783(0.371, 1.653)	0.755 (0.275, 2.076)
	48–57	16(42.1)	22(57.9)	0.727(0.337, 1.571)	0.666 (0.245, 1.811)
	58–65	8(42.1)	11(57.9)	0.727(0.266, 1.985)	0.370 (0.110, 1.241)
Sex	Female	67(58.3)	48(41.7)	2.265(1.368, 3.751)	3.177 (1.671, 6.038)*
	Male	53(38.1)	86(61.9)	1	1
Marital Status	Single	45(46.4)	52(53.6)	1	1
	Married	62(45.6)	74(54.4)	0.968 (0.574, 1.633)	0.717 (0.378, 1.363)
	Divorced	9(64.3)	5(35.7)	2.080 (0.650, 6.660)	1.740 (0.438, 6.913)
	Widowed	4(57.1)	3(42.9)	1.541(0.327, 7.254)	1.294 (0.191, 8.754)
Residence	Urban	54(49.1)	56(50.9)	1	1
	Rural	66(45.8)	78 (54.2)	0.877 (0.534, 1.443)	0.758 (0.394, 1.461)
Educational Status	Unable to read and write	41(56.2)	32(43.8)	1	1
	Able to read and write	41(44.1)	52(55.9)	0.615(0.332, 1.141)	0.563 (0.244, 1.301)
	Preparatory School	19 (41.3)	27(58.7)	0.549(0.260, 1.159)	0.350 (0.087, 1.403)
	College and above	19(45.2)	23 (54.8)	0.645(0.300, 1.383)	0.500(0.163, 1.534)
Occupational Status	Governmental	26(51.0)	25(49.0)	1	1
	Merchant	21(45.7)	25(54.3)	0.808 (0.363, 1.795)	0.566 (0.172, 1.869)
	Student	19(45.2)	23 (54.8)	0.794 (0.350, 1.802)	1.724 (0.374, 7.945)
	Farmer	32 (45.7)	38(54.3)	0.810 (0.393, 1.668)	0.914 (0.315, 2.647)
	Housewife	18 (47.4)	20(52.6)	0.865 (0.373, 2.006)	0.571(0.182, 1.794)
	Other	4(57.1)	3(42.9)	1.282 (0.260, 6.315)	1.035 (0.124, 8.665)
Plasmodium Species	*P. falciparum*	83 (57.6)	61(42.4)	2.671(1.512, 4.719)	2.638 (1.259, 5.527)*
	Mixed	10 (33.3)	20 (66.7)	0.981(0.403, 2.389)	0.597 (0.209, 1.709)
	P. vivax	27(33.8)	53(66.3)	1	1
Parasitemia Level	High	23(46.0)	27(54.0)	0.681(0.348, 1.336)	0.572 (0. 252, 1.296)
	Moderate	37(38.5)	59 (61.5)	0.502(0.287, 0.878)	0.576 (0.283, 1.172)
	Low	60 (55.6)	48 (44.4)	1	1
Blood Group	A	62 (68.1)	29(31.9)	2.915 (1.440, 5.900)	2.746 (1.195, 6.309)*
	B	27(35.1)	50(64.9)	0.736 (0.358, 1.516)	0.862 (0.371, 2.003)
	AB	9(26.5)	25(73.5)	0.491(0.192, 1.256)	0.481(0.166, 1.396)
	O	22(42.3)	30(57.7)	1	1
Rh Type	Rh (+)	106(46.9)	120(53.1)	1	1
	Rh (-)	14(50.0)	14(50.0)	1.132 (0.516, 2.483)	0.811 (0.310, 2.123)

Note: *Significantly associated at P value of <0.05.
Abbreviations: N, Number; *P. falciparum, Plasmodium falciparum*; *P. vivax, Plasmodium vivax*; Mixed, An infection of *P. falciparum and P. vivax*; COR, Crude Odds Ratio; AOR, Adjusted Odds Ratio; CI, Confidence Interval; Rh (+), Rh positive; Rh (-), Rh negative.

000 cells/μL was used to define thrombocytopenia, and 67.7% of study participants had thrombocytopenia. Parasitemia level and *P. falciparum* were identified as associated factors of thrombocytopenia among malaria-infected patients in association with ABO blood groups.

The present study showed significantly varied degrees of malaria infection among the ABO blood groups (P = 0.003). The highest (35.8%) malaria infection was observed among individuals with A (35.8%), followed by B (30.3%), and O (20.5%) blood groups, while individuals with AB (13.4%) blood groups were the least affected. This result is in agreement with the findings done in Sri Lanka,[32] Sudan,[33] and across different corners of Ethiopia.[19,22,34] However, this study was contrary to the findings conducted in China,[35] Burkina Faso,[36] Ghana[37] and Nigeria[38] which reported malaria infection was generally higher in O blood groups than other blood groups. This discrepancy in results could due to

Table 7 Logistic Regression Analysis of Associated Factors with Thrombocytopenia Among Malaria-Infected Adult Patients at Jinella Health Center, Harar, Eastern Ethiopia from July 10, 2022-January 10, 2023 (n = 254)

Variables	Category	Thrombocytopenia		95% CI	
		Yes N (%)	No N (%)	COR	AOR
Age in years	18–27	53(61.6)	33(38.4)	0.574 (0.189, 1.740)	0.996(0.271, 3.663)
	28–37	53(75.7)	17(24.3)	1.113(0.350, 3.545	3.565(0.889, 14.291)
	38–47	28(68.3)	13(31.7)	0.769(0.228, 2.592)	2.348(0.528, 10.435)
	48–57	24(63.2)	14(36.8)	0.612(0.182, 2.064)	1.846(0.431, 7.915)
	58–65	14(73.7)	5(26.3)	1	1
Sex	Female	84(73.0)	31(27.0)	1.570(0.918, 2.687)	1.723(0.869, 3.416)
	Male	88(63.3)	51(36.7)	1	1
Marital Status	Single	69(71.1)	28(28.9)	1	1
	Married	91(66.9)	45(33.1)	0.821(0.466, 1.445)	0.572(0.283, 1.157)
	Divorced	8(57.1)	6(42.9)	0.541(0.172, 1.702)	0.289(0.074, 1.133)
	Widowed	4(57.1%)	3(42.9)	0.541(0.114, 2.575)	0.600(0.091, 3.977)
Residence	Urban	75(68.2)	35(31.8)	1	1
	Rural	97(67.4)	47(32.6)	0.963(0.566, 1.638)	0.928(0.462, 1.865)
Educational Status	Unable to read and write	52(71.2)	21(28.8)	1	1
	Able to read and write	64(68.8)	29(31.2)	0.891(0.456, 1.742)	0.743(0.303, 1.820)
	Preparatory School	28(60.9)	18(39.1)	0.628(0.288, 1.370)	1.147(0.239, 5.504)
	College and above	28(66.7)	14(33.3)	0.808(0.357, 1.829)	0.907(0.279, 2.952)
Occupational Status	Governmental	37(72.5)	14(27.5)	1	1
	Merchant	35(76.1)	11(23.9)	1.204(0.482, 3.006)	1.196(0.327, 4.380)
	Student	23(54.8)	19(45.2)	0.458(0.193, 1.087)	0.638(0.132, 3.089)
	Farmer	40(57.1)	30(42.9)	0.505(0.232, 1.096)	0.613(0.198, 1.904)
	Housewife	31(81.6)	7(18.4)	1.676(0.601, 4.671)	1.176(0.327, 4.229)
	Other	6(85.7)	1(14.3)	2.270(0.250, 20.582)	1.837(0.142, 23.702)
Plasmodium Species	*P. falciparum*	119(82.6)	25(17.4)	6.120(3.301, 11.346)	8.030(3.534, 18.248)*
	Mixed	18(60.0)	12(40.0)	1.929 (0.821, 4.529)	2.049(0.742, 5.656)
	P. vivax	35(43.8)	45(56.3)	1	1
Parasitemia Level	High	42(84.0)	8(16.0)	2.736(1.165, 6.428)	4.396(1.569, 12.318)*
	Moderate	59(61.5)	37(38.5)	0.831(0.469, 1.472)	1.251(0.582, 2.693)
	Low	71(65.7)	37(34.3)	1	1
Blood group	A	67(73.6)	24(26.4)	1.478(0.707, 3.090)	0.983(0.403, 2.402)
	B	47(61.0)	30(39.0)	0.829(0.399, 1.725)	1.040(0.428, 2.526)
	AB	24(70.6)	10(29.4)	1.271(0.500, 3.230)	0.968(0.317, 2.953)
	O	34(65.4)	18(34.6)	1	1
Rh Type	Rh (+)	153(67.7)	73(32.3)	1	1
	Rh (-)	19(67.9)	9(32.1)	1.007(0.435, 2.335)	1.187(0.432, 3.263)

Note: *Significantly associated at P value of <0.05.
Abbreviations: N, Number; *P. falciparum, Plasmodium falciparum; P. vivax, Plasmodium vivax*; Mixed, an infection of *P. falciparum and P. vivax*; COR, Crude Odds Ratio; AOR, Adjusted Odds Ratio; CI, Confidence Interval; Rh (+), Rh positive; Rh (-), Rh negative.

mutations in the genetic mechanisms (A, B, and H carbohydrate antigens), which can regulate protein activities during infection and antibodies against the malaria parasite.[39,40]

A higher proportion of malaria was found to be Rh-positive (89.0%) individuals than Rh-negative individuals, even though no statistically significant association was seen as similar studies reported in India,[41] Senegal,[42] and Nigeria.[43,44] It is uncertain how the Rh- blood group could work as a protective or risk factor for malaria. However, the Rh-blood group changes the parasites' adhesion properties to the RBC membrane and sequesters itself in less suitable areas for malaria infection.[45]

In this study, the overall anemia prevalence was 47.2%. Blood group A malaria-infected patients were 2.75 times more likely to develop anemia than blood group O patients. This finding was supported by some studies conducted in

India,[46] Nigeria,[47] and Ethiopia.[21] The high risk of anemia observed in malaria patients with blood group A might be attributed to the presence of specific receptors (band 3 and glycophorin A) and glycosylated adhesive molecules, which assist in the fast invasion of RBCs by *P. falciparum*.[48]

Whereas the current study disagreed with the results of the study conducted in Colombia[49] that reported a more frequent occurrence of severe anemia among individuals with blood group O. Additionally, blood group AB subjects had the least anemia, in contrast to a study conducted in Cameroon,[50] which reported the most anemic blood group. This inconsistency might be attributed to the presence of A and B antigens on the surface of RBC; cytoadherence, hence rosetting and sequestration, is increased in individuals with blood groups A and B.[51] Another possible explanation may be due to the presence of sialic acid and galactose in the carbohydrate structure of glycophorin A or B, which are the major requisites for the entrance of *P. falciparum* merozoites into human RBC.[52]

The odds of being anemic among female malaria-infected patients were 3.2 times higher than those of their male counterparts. This finding was supported by a study done in India,[53] and Cameroon.[54] The possible explanation might be due to chronic blood loss and abnormal menstrual function among female participants.[55] Malaria patients infected with *P. falciparum* were 2.64 times more likely to develop anemia than those infected with *P. vivax*. This result was in agreement with studies conducted in Colombia[49] and Ethiopia.[21] But in contrast with a study conducted in Australia.[56] This discordant result of anemia in *plasmodium species* might be multifactorial and incompletely understood. But in *P. falciparum*, RBC sequestration reduces the proportion of parasitized RBC that traverse the spleen, while the increased deformability of infected RBC in *vivax* malaria may limit the proportion of RBC that are removed during passage through the splenic microcirculation.[57,58]

In this study, the overall prevalence of thrombocytopenia was 67.7%. The highest prevalence of thrombocytopenia was detected among study participants belonging to blood group A (39.0%), followed by blood group B (27.3%), while its distribution was lower in subjects with AB (14.0%) blood groups. The most severe thrombocytopenia was detected in individuals with blood group B (39.6%). However, the highest prevalence of moderate and mild thrombocytopenia was found among blood group A participants, with a prevalence of 22 (36.7%) and 36 (42.9%), respectively. According to the current study, no significant association was found (p = 0.319) between the blood group and thrombocytopenia, and all ABO blood groups showed similar falls in platelet levels, suggesting that thrombocytopenia was not affected by the ABO blood groups. Studies from India,[59] and Pakistan[60] showed similar findings.

Patients with *P. falciparum* malaria were 8.03 times more likely to develop thrombocytopenia than those with *P. vivax* malaria. This result was supported by studies conducted in India,[61] Indonesia,[62] Pakistan,[63] and Sudan.[64] The pathogenic mechanisms by which platelets mediate disease severity in patients with *P. falciparum* malaria remain to be delineated. However, a study has shown that the platelets of *P. falciparum* patients express Toll-like receptors (TLRs), which release prepared inflammatory mediators.[65] Another reason for thrombocytopenia in malaria infections, particularly those brought on by *P. falciparum*, could be the immune-mediated degradation of circulating platelets.[66]

Malaria-infected patients with a high parasitemia level were 4.40 times more likely to develop thrombocytopenia than malaria-infected patients with a low parasitemia level. This result was supported by studies done in Thailand,[67] Senegal,[68] and Sudan.[64] Evidence suggests that malaria thrombocytopenia at high parasitemia was found to significantly lower the PLT count.[69] As malaria parasitemia increases, platelets play a critical role in enabling the cytoadherence of malaria-infected erythrocytes (IEs) to ECs, and Von Willebrand factor (VWF) then promotes this platelet-mediated IE cytoadherence during the malaria infection.[70] Finally, platelets enhance IE clumping and sequestration, which further contributes to its decrease during the infection.[71]

Changes in WBC count are less dramatic than in other blood cell series and have been controversial. Of the total number of study participants, 81 (31.9%) had leukopenia. This study was in accordance with the studies conducted in Colombia[72] and Pakistan.[73] This could be a result of the sequestration of leukocytes, accelerated destruction, or decreased production.[74] The highest number of leukopenia was observed among blood group A (39.5%), followed by blood group O study participants (25.9%), in contrast with the studies conducted in India[59] and Pakistan[60] which were higher in B and AB blood groups, respectively. This discrepancy in results across different studies might be due to the methods they used for the determination of WBC counts and statistical tests that assume Gaussian distributions of data.[75]

Regarding leukocytosis, 33 (13.0%) of the study participants had leukocytosis. The same results were reported from Burkina Faso[76] and Thailand.[67] The highest number of leukocytosis was detected in blood group B (33.3%), similar to

a study conducted in Pakistan.[60] This might be attributed to the increased production of leukocytes at the onset of an infection to engulf the invading parasite.[77] The ABO blood group and WBC alterations among malaria-infected patients did not have a statistically significant correlation.

Strengths and Limitations of the Study

The study's strength is that all hematological abnormalities were detected. However, due to its cross-sectional nature, the study is unable to demonstrate the temporal relationship between the hematological abnormalities and the identified related factors. Additionally, the study did not apply any reliable assays, like PCR, that can distinguish distinct malaria parasites with comparable characteristics.

Conclusion

Of the examined malaria-infected adult patients, almost half, more than half, one-third, and almost one-eighth of the patients had anemia, thrombocytopenia, leukopenia, and leukocytosis, respectively. There was a significant association between the ABO blood groups and susceptibility to malaria-related anemia. Being female, having an "A" blood group, and being infected with *P. falciparum* were identified as associated factors of anemia among malaria-infected patients about ABO blood groups. On the other hand, parasitemia level and *P. falciparum* were identified as associated factors of thrombocytopenia among malaria-infected patients in relation to ABO blood groups. There was no significant association between blood groups and susceptibility to malaria-developing thrombocytopenia, leukopenia, or leukocytosis. Therefore, there should be routine screening for anemia, thrombocytopenia, and WBC alterations for all malaria patients.

Abbreviations

BF, Blood Film; EDTA, Ethylene Diamine Tetra Acetic Acid; ECs, endothelial cells; Hgb, Hemoglobin; ICAM-1, Intravascular cell adhesion molecule-1; IEs, Infected erythrocytes; RBC, Red Blood Cell; *P. Falciparum, Plasmodium Falciparum*; PLT, Platelet; *P. vivax, Plasmodium Vivax*; VWF, Von Willebrand factor; WBC, White Blood Cell.

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Author Contributions

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