Hematological parameters of malaria infected adult patients in Raya Alamata Hospital, Northeast Ethiopia.

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Research

Keywords: Malaria, Malaria parasitaemia, Hematological parameters, Ethiopia

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Abstract

Background: Hematological changes are the most common complications encountered in malaria and they play a major role in malaria pathology. Hematological changes like red blood cells, platelets and leukocytes have been observed in patients with malaria. Therefore, this study aimed to compare hematological parameters of malaria infected adult patients in Alamata Hospital, Northeast Ethiopia from February 01-April 30, 2019.

Methods: Comparative cross sectional study was used to compare the hematological parameters on a total of 238 study participants, consisting of 119 malaria infected patients and 119 malaria negative controls at Raya Alamata Hospital. Malaria diagnosis was done based on thick and thin blood films microscopy. Hematological parameters were determined by using an automated, DiRUi BCC/3000B hematology analyzer. Malaria parasite density was determined by counting the asexual parasites against 200 WBCs, and then calculated by using the standard formula. Data for the different hematological parameters were expressed as mean (±SD). Independent t-test was used for comparison the hematological parameters between the two groups. Binary logistic regression model was constructed for categorical dependent variables to see associations between predictors and outcomes.

Results: In current study parameters like red blood cells count, hemoglobin, mean corpuscular volume, platelets count, and eosinophil counts were significantly lowered in malaria-infected patients than the controls. The prevalence of anemia and thrombocytopenia in malaria patients was 39.5% and 56.3%, respectively. Being female and malaria parasitaemia were found significantly associated with thrombocytopenia. The odds of developing thrombocytopenia with high malaria parasitaemia were 8.4 times more likely develop thrombocytopenia.

Conclusion: Anemia and thrombocytopenia were the two common hematological abnormalities seen in malaria patients. The platelet count during malaria infection was inversely correlated with the parasitaemia. Malaria patients should be checked for the presence of hematological abnormalities such as anemia and have to be managed for those abnormalities. Keywords: Malaria, Malaria parasitaemia, Hematological parameters, Ethiopia

Background

Malaria is the major public health problem which is one of the major causes of mortality and morbidity in Sub-Sahara Africa (1–3). It is endemic in Nigeria, Democratic Republic of Congo, Uganda, and Ethiopia and as such approximately half of global malaria case occurs in these regions. (2, 4). Malaria is a disease caused by protozoan parasites of genus Plasmodium. The four Plasmodium species well known to cause human malaria are; Plasmodium falciparum (PF), Plasmodium vivax (PV), Plasmodium ovale (PO), and Plasmodium malariae (PM). However, PF is responsible for most malaria deaths (5).

Malaria is characterized by fever, anemia and splenomegaly (6). Anopheles mosquito that carries the parasite deposits the sporozoites on the host, which then invades the liver and then the red blood cells (RBCs), thereby, predisposing the individual to intermittent shivering, pyrexia, sweating and spleen

enlargement (7). Hematological changes are the most common complications encountered in malaria infection and they play a major role in malaria pathophysiology. Hematological changes can occur in the major cell lines, such as RBCs, leukocytes or white blood cells (WBCs) and platelets (8). Anemia, thrombocytopenia, splenomegaly, mild-to-moderate atypical lymphocytosis and infrequently disseminated intravascular coagulation (DIC) (9), have been reported invariably accompanied with malaria infection.

Pathophysiology of malaria on hematological parameters change is caused by several factors including parasite proteins on the surface of infected RBCs. Proteins of the plasmodium falciparum (PF) RBC membrane protein 1 (RBCMP1) family attaches to the endothelium and mediate the adhesion of parasite through specific binding to multiple cell receptors (10, 11). Intercellular adhesion molecule-1 (ICAM-1), CD36, E-selectin, neural cell adhesion molecule are the major adhesion molecules for the attachment of malaria parasites. This attachment can lead to endothelial activation, pro-inflammatory and pro-coagulant responses and causing microvascular obstruction, hypoxia, and inflammation (12). Release of a variety of toxins triggers the activation of host immune factors like cytokines, tumor necrosis factor alpha (TNFα), pro-inflammatory interleukins, oxygen free radicals and nitric oxide, which result in damage to host endothelium and tissues (13). According to the 2018 World Health Organization (WHO) report, 219 million cases and 435,000 estimated deaths were recorded from malaria globally. Around 80% of global malaria deaths were predominantly occurred in 17 countries in the WHO African region and India. For example, an estimated 3.5 million malaria cases were reported from 10 African countries with highest burden of malaria in the year 2017 (14).

Malaria is a major public health problem in Ethiopia in which more than 50 million people in the country live in areas at risk of malaria transmission. It is also estimated that 9 million malaria cases occur annually throughout the country. PF and PV are the two most common malarial

parasites in the country (15).

Entry of PF into erythrocytes usually go to a markedly rises in secretion of inflammatory cytokines, endothelial cell activation, initiation of the coagulation pathways, and sequestration of parasitized RBCs (16). Reduction in platelets, hematocrit (Hct), haemoglobin (Hgb), and elevation in white blood cell (WBC) and erythrocyte sedimentation rate (ESR) were manifested in malaria patients (17).

Malaria parasites targeted RBCs and gradually cause anemia. Anemia is the major manifestations of malaria that results from RBCs destruction after the parasites invade and develop within the cells. In the case of acute malaria infection, non-parasitized RBCs may also undergo hemolysis (18). Thrombocytopenia is also a typical characteristic of malaria, especially with PV malaria (19). Other hematological changes include neutropenia, eosinophilia, neutrophilia and monocytosis were commonly observed in studies conducted in different parts of the world (20), Africa (21) and Ethiopia (22). So, determination of the hematological changes enables the clinician to establish an effective and early therapeutic intervention in order to prevent the occurrence of major complications in those malaria patients. These parameters are measurable indices of blood that serve as a marker for disease diagnosis (9).

Malaria could lead to changes in haematological status alongside with other conditions such as anaemia, thrombocytopenia and leukocytosis, leukopenia, lymphocytosis, monocytosis, eosinophilia and neutrophilia (23). Thrombocytopenia is among the major haematological aberrations frequently observed among malaria patients (24). Variations in haematological parameters may be influenced by any disease situation such as malaria complications and pathogenesis (25, 26), malaria parasitaemia cause anaemia by imposing a degenerative impact that suppresses bone marrow functionality (27). Although malaria is known to be the most common public health problems in Ethiopia (28), limited study that evaluates the extent of hematological changes in malaria infected individuals particularly in this study area has been done. Additionally, the associations of thrombocytopenia and leukopenia with malaria have not been well documented. Moreover, most researchers did not give emphasizes on WBC differentials in these patients. Therefore, this study was designed to compare hematological changes in malaria infected adult patients and apparently healthy controls in Raya Alamata Hospital, Northeast Ethiopia.

Methods

Study area and period

A hospital based comparative cross sectional study was conducted in Raya Alamata Hospital from February 01 to April 30. Alamata town is located at 600 Km far from Addis Ababa, Ethiopia in northeast direction and situated at an elevation of 1521.95 m above sea level.

Study population, design, sample size determination and sampling technique

All acute febrile patients clinically suspected of malaria adult patients attending Alamata Hospital during the study period, and age and gender matched blood donors were considered as a source population. Those all adult patients with blood film microscopy positive malaria patients satisfying inclusion criteria attending the hospital during the study period were used as a study population. All adult patients confirmed for malaria infection and who were consented to participate in the study was included in malaria infected group. Additionally, age and gender matched apparently healthy blood donors during the study period were included in the control group. On the other hand, adults having a history of chronic disease like hypertension, cardiac disease, and diabetes mellitus, and adults who were positive for HIV, hepatitis B surface antigen test and hepatitis C virus, intestinal parasite (s), patients on antimalarial treatment, patients co- infected with Borrelia or Babisia, pregnant and lactating women and those patients younger than age 18 and older than 65 years old were excluded from the study.

Comparative cross sectional study design was used in this study. Sample size was calculated by using a double population proportion formula for comparison of two populations mean obtained from previously conducted study in Metema, Ethiopia by the year 2016 (29). Accordingly, a total of 238 (119 malaria positive and 119 screened blood donor) participants were included in the study. Systematic random sampling technique was used to select the study participants. First the total frame size (N) was recorded from the hospital, those who were infected with malaria in previous year at the same month in which the study was conducted. Then, the number of sampling intervals (K) was determined by dividing total malaria infected patients in previous year (N = 291) to calculated sample size (n = 119) (K = 291/119 = 2). After this, the first

patient (the first sampling unit) was randomly selected between 1 and K, and the next participants were selected every K^{th} (2) unit until the required sample size (119) obtained.

Results

Socio-demographic characteristics of the study participants

A total of 238 malaria-infected adult patients and healthy controls were included in this study. Majority of the study participants (86 (72.3%)) were males for both cases and controls. Out of the total study participants, around 70 (58.8%) and 33 (27.7%) were from rural residence for cases and controls, respectively. Regarding to marital status of the study participants, 54 (45.4%) of the malaria infected participants were unmarried. About 43 (36.1%) malaria infected patients were able to read and write. Forty (33.6%) of the study participants among the malaria infected group were farmers (**Table 1**).

Table 1: Socio-demographic characteristics of the study participants at Alamata Hospital, northeast Ethiopia, 2019.

Variables	Category	Cases (n (%))	Controls (n (%))
Age in years	18-27	57 (47.9)	57 (47.9)
	28-37	32 (26.9)	35 (29.4)
	38-47	11 (9.2)	13 (10.9)
	48-57	14 (11.8)	10 (8.4)
	58-65	5 (4.2)	4 (3.4)
Gender	Male	86 (72.3)	86 (72.3)
	Female	33 (27.7)	33 (27.7)
Marital status	Single	54 (45.4)	53 (44.5)
	Married	51(42.9)	60 (50.4)
	Divorced	8 (6.7)	4 (3.4)
	Widowed	6 (5.0)	2 (1.7)
Residence	Urban	49 (41.2)	86 (72.3)
	Rural	70 (58.8)	33 (27.7)
Educational status	unable to read and write	34 (28.6)	10 (8.4)
	able to read and write	43 (36.1)	24 (20.2)
	preparatory school	24 (20.2)	24 (20.2)
	College and above	18 (15.1)	61(51.3)
Occupational status	Governmental	14 (11.8)	59 (49.6)
	Merchant	17 (14.3)	11(9.2)
	Student	24 (20.2)	25 (21.0)
	Farmer	40 (33.6)	11(9.2)
	Housewife	19 (16.0)	11(9.2)
	Other	5 (4.2)	2 (1.7)

Clinical characteristics of *malaria* case study participants

Out of the total 119 malaria infected study participants, 52 (43.7%), 50 (42.0%) and 17 (14.3%) were positive for PV, PF and mixed infection (PV and PF), respectively. Around 86 (72.3%) of the case were males and of them around 41 (47.7%), 30 (34.9%) and 15 (17.4%) patients were infected by PV, PF and mixed infection,

respectively. Twenty (60.6%) of female patients were infected by PF and only 2 (6.0%) patients were mixed infection. Among the female participants, 20 (60.0%) were infected by PF. Around 36 (41.9%) of males and 14 (42.4%) of females had moderate parasitaemia. In the present study, headache was the most common (98.3%) clinical manifestation among the cases followed by fever (97.5%) (**Table 2**).

Table 2: Clinical characteristics of cases (n=119) at Alamata Hospital, northeast Ethiopia, 2019.

Variable	Category	Frequency	Percentage
Fever	Yes	116	97.5
	No	3	2.5
Headache	Yes	117	98.3
	No	2	1.7
Nausea	Yes	69	58.0
	No	50	42.0
Dizziness	Yes	83	69.7
	No	36	30.3
Vomiting	Yes	53	44.5
	No	66	55.5
skeletal pain	Yes	100	84.0
	No	19	16.0
abdominal pain	Yes	34	28.6
	No	85	71.4
Diarrhea	Yes	18	15.5
	No	101	84.9
Malaria species	PF	50	42.0
	PV	52	43.7
	Mixed*	17	14.3

Mixed*= PV and PF mixed malaria infection

Hematological profiles according to plasimodium species

In this study, the average Hgb concentration in control and malaria subjects were 15.04 g/dl and 12.80 g/dl, respectively. The prevalence of anemia among the cases was 47 (39.5%) with the mean Hgb value of

12.80±2.42 g/dl. Of the anemic individuals, 33 (70.2 %), 11 (23.4%) and 3 (6.4%) were mildly, moderately and severely anemic, respectively. PF, PV and mixed infections were responsible for the occurrence of 16 (34.04%), 23 (48.94%) and 8 (17.02%) of anemia cases, respectively. Three (6.4%) malaria patients were found severely anemic accounted by2 (66.7%) mixed and 1(33.3%) PF infection. On the other hand, 11(23.4%) malaria patients were found moderately anemic due to 5 (45.5%), 4 (36.4%) and 2 (18.2%) PF, PV and mixed infection, respectively. Whereas, mild anemia was found in 33 (70.2%) cases with 18 (54.5%), 10 (30.3%) and 5(15.2%) PV, PF and mixed infections, respectively. From the total anemic patients, 18 (38.3%), 23 (48.9%) and 6 (12.8%) showed high, moderate and low parasitaemia, respectively (Table 3).

The overall prevalence of thrombocytopenia in this study was 67 (56.3%) among the cases with mean platelet count of (153.93±89.345)x10^3/ul. Of them, 32 (47.8%), 26 (38.8%) and 9 (13.4%) were mildly, moderately and severely thrombocytopenic, respectively. Of the thrombocytopenic patients, around 31 (46.3%), 27 (40.3%) and 9 (13.4%) were due to PF, PV and mixed infections, respectively. Four (44.4 %) patients with PV, 3 (33.3%) patient with PF and 2 (22.2%) patients with mixed infection had severe thrombocytopenia. Whereas moderate thrombocytopenia was found in 50.0%, 42.3% and 7.7% of PF, PV and mixed infection, respectively. Mild thrombocytopenia was also found in 46.9%, 37.5% and 15.6% of patients with PF, PV and mixed infection, respectively. Of the malaria thrombocytopenia, about 38 (56.7%), 23 (34.3%) and 6 (9.0%) patients showed high, moderate and low parasitaemia, respectively. On the other hand, most of the patients suffering from malaria had normal total leukocyte count (4000-11000 WBCs/μL). However, leukocytosis (WBC > 11´10^3 / μL) was observed in 3 (2.52%), 4 (3.36%) and 1 (0.84%) patients suffering from PF, PV and mixed infection, respectively. Twenty-seven malaria patients (22.7%) had leukopenia (WBC < 4´10^3 / μL). Of whom, 20 (74.1%), 5(15.5%) and 2 (7.4%) had suffer from PF, PV and mixed malaria infections, respectively (Table 3).

Table 3: Hematological changes in malaria patients (n = 119) at Alamata Hospital, Northeast Ethiopia, February – April, 2019.

Hematological alterations	Malaria species			
	PF= n (%)	PV = n (%)	Mixed* = n (%)	Total = n (%)
Anemia	16 (34.04%)	23 (48.94%)	8 (17.02%)	47 (39.5%)
Thrombocytopenia	31 (46.3%)	27 (40.3%)	9 (13.4%)	67 (56.3%)
Leukopenia	20 (74.1%)	5 (18.5%)	2 (7.4%)	27 (22.6%)
Leukocytosis	3 (37.5%)	4 (50.0%)	1 (12.5%)	8 (6.72%)

Mixed*= PV and PF mixed malaria infection

Comparisons of hematological parameters among cases and controls

There was a significant mean difference with respect to RBC, Hgb, HCT, MCH, MCHC, and RDW. The mean of Hgb in patients with malaria (12.81 ±2.42) was significantly lower than non-malaria (15.04 ±1.90) groups (P < 0.0001). The mean of RBCs count was significantly lower in malaria patients (4.16× 10^6/µL) than non-

malaria (4.77× 10^6/μL) groups (P <0.0001). The mean of HCT, MCV and MCH in malaria patients were significantly lower than non-malaria group (P value < 0.001). Conversely the mean value of MCHC was significantly higher in malaria group than non-malaria group. The mean value of RDW was significantly increased in malaria groups than control group (P < 0.0001) (**Table 4**).

However, no significant difference observed in total leukocyte count, lymphocyte, monocyte and basophil. There was no significant difference in total WBCs count between malaria patients and control groups. But neutrophil counts were significantly increased in malaria infected patients as compared with non-malaria group. The mean of neutrophil count was significantly higher in patients with malaria infected groups (63.43±14.12) than non-malaria (56.36±7.88) groups. Another leukocyte component which was significantly associated with malaria was eosinophil. The mean of eosinophil count in malaria group was (2.30±1.14) and significantly decreased than non- malaria health controls (3.02±.90). The mean platelet count in malaria infected group (153.93× 10^3/μL) was significantly lower than non-malaria (284 × 10^3/μL) group. On the other hand, MPD and PMV were found significantly increased in malaria infected groups (**Table 4**).

Table 4: Mean values (±SD) of hematological parameters among malaria infected patients and control group at Alamata Hospital, northeast Ethiopia, 2019.

Parameters	Malaria group (mean ± SD)	Controls group (mean ± SD)	*P*-value
WBC(x10^3/ul)	7.34±2.96	6.462±1.72	.261
RBC (x10^6/ul)	4.16±.72	4.8± .52	< 0.001
Platelet (x10^3/ul)	153.93±89.35	285.0±75.56	< 0.001
Hgb (g/dl)	12.81±2.42	15.10 ±1.90	< 0.001
HCT (%)	34.28±6.33	41.00 ±5.64	< 0.001
MCV(fl)	80.94±4.57	87.88±4.26	< 0.001
MCHC(g/dl)	38.91± 4.13	36.71±1.39	< 0.001
Neutrophil (%)	63.43± 14.12	56.36±7.88	< 0.001
Monocyte (%)	5.27±3.64	5.30±1.45	0.95
Lymphocyte (%)	28.34±11.44	30.31± 6.14	0.14
Eosinophil (%)	2.29 ±1.14	3.02±0.90	< 0.001
Basophil (%)	0.20 ± .23	0.25±.19	0.098
RDW (%)	14.73 ±1.51	13.52±0.60	< 0.001
PDW (%)	19.95 ±2.96	15.47±1.60	< 0.001
MPV(fl)	9.89 ±2.48	8.53±1.48	< 0.001

Abbreviation: MPV: Mean Platelet Volume; PDW: Platelet distribution width; RDW: Red Cell Distribution Width; WBC: white blood cell.

Differential leukocyte counts in malaria infected patients

The total leukocyte count was normal in 60 (50.4%) whereas differential leukocyte count showed normal neutrophil count in 63(52.9%), normal lymphocytes in 51(42.9%), normal monocytes in 58(48.7%), normal basophil in 73 (61.3%) and normal eosinophils in 63 (52.9%) patients. Monocyte as well as neutrophils were increased in 14 (11.8%) and 9 (7.6%) cases, respectively. However, lymphopenia was present in 18 (15.1%) cases *(Table 5).*

Table 5: Differential leukocyte count alterations in malaria infected patients at Alamata Hospital, northeast Ethiopia, 2019.

Differential Count	Low No. (%)				High No. (%)				RR (34)
	Pf	Pv	Mixed	Total	Pf	Pv	Mixed	Total	
Neutrophil	2(28.6)	3(42.9)	2(28.6)	7` (5.90)	7 (53.8)	2 (15.4)	4(30.8)	13 (10.92)	(40-80)
Lymphocyte	13(52.0)	7(28.0)	5(20.0)	25 (21.0)	10 (50.0)	7 (35.0)	3(15.0)	20 (16.80)	(20-40%)
Monocyte	3(60.0)	2(40.0)	0(00)	5 (4.2)	3 (27.30)	6 (54.5)	2(18.2)	11 (9.24)	(2-10%)
Eosinophil	2(100.0)	0(00)	0(00)	2 (1.70)	1 (100.0)	0 (00)	0(00)	1 (0.84)	(1-6%)
Basophil	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	(<1%)

RR =Reference range, Diff. Count=Differential count**,** NR=Normal range, Neut =Neutrophil, Lymp =Lymphocyte, Mono =Monocyte, Eosin =Eosinophil, Baso =Basophil, Low No = Low number, High No = High number, R=Reference

Discussion

Hematological changes are considered as a hallmark of malaria infection. Most of the time, these changes in malaria infection may be as a result of the higher levels of parasitaemia (35).

In the current study, the prevalence of anemia was 39.5% with a 30.8% – 48.2% 95% CI. Comparable result was found in a study conducted in western Maharashtra with the prevalence of 35% (36). However, the prevalence of anemia found in this study was lower than similar studies conducted in Iran (65.5%) (8), Maharashtra, India 71% (37), Karamsad, India (49.01%) (37, 38) and Durban, South Africa (63.0%) (21). This

discrepancy might be due to seasonal variation for plasmodium species, geographical location, method of study design and differences in total prevalence of malaria in the country.

On the other hand, the prevalence of anemia found in this study was higher than similar studies conducted in South India (15.8%) (39). This discrepancy might be due to a study done only on a single plasmodium species and sample size variation. The occurrence of severe malarial anemia might be also due to hemolysis of parasitized RBCs, increased destruction of parasitized and un-parasitized erythrocytes (immune-mediated lysis, phagocytosis, splenic sequestration (40, 41).

In the current study, the prevalence of thrombocytopenia has been found 56.3% (95% CI = 47.4–65.2). Comparable result was found a study conducted in Pakistan 53% (42), Saudi Arabia 57% (43), Durban, South Africa (50.0%) (21) and Hissar, India 62% with 8.87% mild, 39.52% moderate and 51.62% severe thrombocytopenia (8). But the prevalence of thrombocytopenia found in this study was lower than similar studies conducted in Dammam, Saudi Arabia 67.5% (44), Gujarat, India (82%) (45), Karamsad, India (94.11%) (38), Eastern India 90% (46).

Being female, the odd of developing thrombocytopenia in malaria infection is 3.7 times more likely that of males (AOR = 3.65, CI = 1.238, 10.759). The odds of developing thrombocytopenia in moderate parasitaemia level of malaria infected patients were 94.4% less likely that of high parasitaemia level (AOR = 0.056; CI: 0.014, 0.225). Additionally, the odds of developing thrombocytopenia with low parasitaemia level of malaria infected patients were 98.6% less likely that of high parasitaemia level (AOR = 0.014, CI: 0.002, 0.074).

On the contrary, the prevalence of thrombocytopenia in this study was higher than similar study conducted in Maharashtra, Indonesia (33%) (36), Aden 42.9% (47) and Turkey 47% (48). This discrepancy might be due to the study designs used, sample size variation between different studies and type of study participants. Another possible cause for this discrepancy might be due to splenic pooling of platelets, antibody (IgG) mediated platelet destruction and adenosine diphosphate (ADP) release following the hemolysis of parasitized RBCs (49–51).

In the present study patients with thrombocytopenia were not likely to have an anemia ($r = -0.065$, P value = 0.480) and not correlate with age ($r = 0.024$, P value = 0.795) in contrast with previously reported from Thailand-Myanmar border (34). This difference might be due to the difference in study participants, because the participants of the previous study were included children. The odds of developing thrombocytopenia with high parasitaemia level of malaria infected patients were 8.4 times that of low parasitaemia level. Parasitaemia level in this study has also been shown that the directly relationship with thrombocytopenia ($r = 0.396$, $p < 0.0001$) similar with study conducted in Nigeria (52). This relationship might be due to binding of platelets with infected and an uninfected RBC cells for fighting malaria parasite. This association may result from sensitization induced by parasitized RBCs in platelets, with consequent increase in platelet sensitivity to adenosine diphosphate and higher dense-granule secretion (53).

In the present study sever thrombocytopenia was common in PV malaria in lines with study reported previously in Iran (8). But it was not compatible with the study conducted on malaria parasite density on blood cell parameter by Kotepui. M. This difference might be due to the nature of recrudescence, becoming

dormant at hepatic cell, incapability of laboratory technician to differentiate the species exactly and the quality of Geimsa stain.

With respect to RBC indices (Hct, MCV,RBC and Hgb) were significantly decreased in malaria patients than control group similar to the studies conducted in Thailand (54), Nigeria (55), Uganda, Kampala (49) and Durban, South Africa (21). Mean cell hemoglobin count was non-significantly decreased but Hgb and Hct showed statistically significant decrement in malaria patients in concordance with study conducted in Metema, Ethiopia (22). But MCHC were statistically significant increment in contrast to study conducted in Thailand-Myanmar border (34) and Metema, Ethiopia (22). In contrast Hct, MCV and MCHC were found significantly increased in malaria infected patients than that of controls in a study conducted in Thailand-Myanmar border (34). This discrepancy might be due to the difference in analysis methods, distribution of the data, using of different hematological analyzer machine, reagents and calibration system at the beginning. Another possible reason might be due to the variety of morphological changes exhibited by RBCs malaria- associated changes in composition of plasma, mechanical destruction of infected RBCs, and intravascular hemolysis caused by non-immune destruction of infected RBCs in case of high parasitaemia. This may be also the reason why the rate of RBC production leads to the release of immature RBCs into blood circulation, which may cause an increase in the values of MCHC in malaria infection.

The current study have been found an increased RDW in malaria infected patients in concordance with previous study conducted in India (56), but in contrast with a study conducted in Thailand-Myanmar border (34). This difference may be attributed to the red cell response to malarial parasite. Comparing to control groups malarial infected patients had higher total WBC count and neutrophil count in contrast with study conducted in south western Nigeria (52) and Metema, Ethiopia (22). Eosinophil was statistically decreased in malarial patients than health controls in line with a study conducted in southern India (57) in contrast with previous study (58). This difference might be due to the method for eosinophil count because in this study eosinophil was counted by manually but in the previous study it was counted by hematology analyzer. Another possible suggestion might be due to acuteness of the infection.

In the present study, total leukocyte count in study participants, nearly 23% of the patients had total leukocyte count $< 4 \times 10^3/\mu L$ (leucopenia), 70.6% had $4–11 \times 10^3/\mu L$ and 6.7% had counts $> 11 \times 10^3/\mu L$ (leukocytosis) similar to the study done in Iran and western India (8, 57). Another congruent study was done in a tertiary care Hospital in Western Maharashtra. In their study, 23% of the patients had leukopenia, 69% had normal levels and 8% of patients had leukocytosis. In contrast with the study done in Chhattisgarh (59).

Leukopenia has seen in the malaria-infected patients which was confirmed by other studies conducted in western Thailand and Thailand-Myanmar border (34, 54) but in contrast with another study that has been reported leukocytosis (60). Although, some controversies appear to exist, there have been reports of leucopenia as well as leukocytosis. Changes in the white blood cell were also less dramatic and there has been conflicted reports regarding these changes, may vary due to variable size and type of cases, variability of the species, geographical differences, and there has been conflicting reports regarding these changes, and cannot be used as a predictor for severity. These variations in the leukocyte numbers is might to be

dependent on many factors including the acuteness of infection, the parasitaemia level, the severity of the disease, state of host immunity to malaria and concurrent infections (57).

The differential white cell counts showed a normal neutrophil count in the majority (83.2%) of cases, which differs from other studies, which reported majority of either neutropenia or neutrophilia among malaria cases conducted in previous (34). This finding was also in contrast with that of previous study which reported that malaria induced changes include a reduction in neutrophil levels (52). This variation might be due to shifting of neutrophils from the circulatory to the marginal pool to sites of inflammation, splenic localization (52), acuteness of malaria and immunity status of the patient. In the current finding both monocyte and lymphocyte have been shown a deviation from normal reference range even if they were not significantly associated.

Basophil was the only WBC subtype without deviation from normal reference range. Similar study was reported in Chhattisgarh (59) that both monocyte and lymphocyte were deviated from the normal reference range. Involvement in immune response against malaria, acuteness of the disease and parasitaemia level might be lead to deviation of those WBC subtype in malaria infection. In this study, the MPV and PDW were significantly increased from control group in agreement with a study conducted in Kasturba Medical College, Mangalore 8.98 ± 0.7813 and 17.48 ± 1.224, respectively. But a study conducted in Iran showed that MPV and PDW were statistically decreased than control groups (8). This discrepancy might be due to the patient status and acuteness of the disease that the bone marrow is rapidly producing platelets. This may be because of older platelets were destroyed, so the bone marrow might be tried to compensate

Conclusions

In this study, the prevalence of thrombocytopenia and anemia were significantly higher in the malaria infected patients than control groups. Mean values of hematological parameters were significantly different in the malaria groups than the controls. Therefore, prediction of the hematological changes enables the clinician to establish an effective and early therapeutic intervention in order to prevent the occurrence of major complications. This may be used in addition to the clinical and microscopic parameters to heighten the suspicion of this disease and prompt initiation of the treatment. Further longitudinal study should be conducted to identify the cause effect relationships. Researchers that are going to be done for the future will be also better if the nutritional statuses of the patient, large sample size and cell morphology are included.

Declarations

Competing interests
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Authors’ contribution
HA, AY and ZG conceived the study, participated in the design and data analysis. HA involved in data acquisition, laboratory work and drafted the manuscript. AY and ZG critically reviewed the research work and

the preparation of manuscript. All authors read and approved the manuscript.

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Abbreviations

CBC: Complete blood count; **Hct**: Hematocrit; **Hgb**: Hemoglobin; **MCV**: Mean Cell Volume **MCH:** Mean Cell Hemoglobin; **MCHC**: Mean Cell Hemoglobin Concentration; MPV: Mean Platelet Volume**; ICAM-1:** Intercellular adhesion molecule-1; **NCAM:** Neural cell adhesion molecule; **PDW: Platelet distribution width; PF:** Plasmodium falciparum; **PV***:* Plasmodium vivax; **RBCMP1**: RBC membrane protein 1; **RDW:** Red cell distribution width; **SOP:** Standard Operating procedure; **TNFa:** tumor necrosis factor alpha; WBC: white blood cell; **WHO:** World Health Organization

Declarations

Ethics approval and consent to participate

Ethical clearance was obtained from School of Biomedical and Laboratory Sciences, Research and Ethical Review Committee College of Medicine and Health Sciences, University of Gondar. Letter of permission to conduct the study was obtained from Alamata Hospital Clinical Director's office. From all study participants, oral and written consent were obtained prior to data collection. Any information concerning the participants was kept confidential and the specimens collected from the participants were only analyzed for the intended purposes. Study participants with abnormal hematological findings were linked to physicians for appropriate treatment.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that there is no competing interest.

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